GELLAN. PHARMACEUTICAL, MEDICAL AND COSMETIC APPLICATIONS

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Ever since it was approved by the FDA as a food additive in 1992, gellan, a polyanionic exopolysaccharide from microbial culture, has become a very important polymer for biological applications. The area of its applications has rapidly expanded in the pharmaceutical and cosmetic fields, food, medicine, tissue engineering and biotechnology. This paper aims to expand the previous review that presents the use of gellan in the food industry and make an overview of other polysaccharide bioapplications. Considering the interest it has attracted in recent years, the paper details the use of gellan in pharmacy, medicine and tissue engineering. Also, other gellan bioapplications in cosmetics, biotechnology, microbiology are concisely presented.

Keywords: gellan, pharmaceutical applications, medical applications, tissular engineering applications, cosmetic applications

INTRODUCTION

Natural polymers are biodegradable, prone to decomposition by chemical or enzyme catalyzed hydrolysis to secondary biocompatible products. This property allows them to be implanted in the human body without the need for subsequent removal by surgery. The drugs incorporated into these biopolymers are delivered by controlled release over a prolonged period of time and their concentration at the target site in the body is maintained within the therapeutic range.[1,2] The release rate of the drugs from the biopolymer can be controlled by a number of factors, such as:

- biodegradation kinetics of polymers;[3]
- physico-chemical properties of polymers and drugs;[4]
- thermodynamic compatibility between polymers and drug;[5]
- the shape of devices for drug delivery.[6,7]

Among natural polymers, polysaccharides are considered to be very important due to their abundence, structural diversity, various properties, and ease of chemical modification for creating new properties. Hydrogels are very important among the biomaterials based on polysaccharides or the combination of the latter with proteins or some synthetic polymers.

Hydrogels, which can be obtained through various physical and chemical methods, are three-dimensional networks capable of absorbing large quantities of water or biological fluids. The functional properties of hydrogels have led to their use in transport applications for drugs, enzymes, cells, nutraceuticals and other biologically active principles. Hydrogels have a porous structure, which can be adjusted by controlling the crosslinking density of the gel matrix.[8] They resemble natural living tissue more than any other biomaterial and the high water content contributes to good biocompatibility. When the polymers transport system phase changes, drug release is initiated, as a response to external stimuli. Hydrogels based on such

polymers can suffer phase transition, volume or sol-gel phase changes depending on the environmental conditions. Consequently, they play an important role because they can determine where and how the active principle is released and the time period when this process occurs.[9,10]

Gellan forms stronger gels (hydrogels) with cations (especially divalent ions).[11,12] The acyl groups that gellan may contain also affect the gel strength.[13]

The present paper aims to review the main bioapplications of this polysaccharide. Although in the last years, several reviews and monographs about this polysaccharide have been published, the originality of our paper consists in the presentation of exclusively its bioapplications in a single material. Also, a chapter has been dedicated to cosmetic applications of gellan, which are mentioned only sparsely in different works.

PHARMACEUTICAL APPLICATIONS

The polymer was initially used as a food ingredient. Due to its functional properties, it is increasingly used for obtaining new transport systems for drugs. Gellan gels are thermoreversible and have a melting temperature of 50 ºC; also they depend on the concentration and the presence of cations to stabilize them, thereby increasing their thermoreversible character.[14]

The patients have shown an increased compliance to gellan due to *in situ* gel formation; therefore it has been investigated for producing transport polymeric systems for ophthalmic drugs with controlled release at the application site. Gellan gel has a low viscosity in the absence of cations. When eye drops solution containing gellan and active drug substances (without cations) are administrated, the sol-gel transition occurs in the presence of tear fluid. The bioavailability of the drug depends on the gel strength. *In vivo* studies have demonstrated that only when the gel strength is within certain limits, the maximum ocular bioavailability is obtained. The contact time of the gel in the eye increases with an increased concentration of gellan. The process of autoclaving, which is used to sterilize the solutions of gellan, could lead to a significant reduction in gel strength in the final product, due to breaking of polymer chains – proportionally with the autoclaving time.[15,16]

The gels formed with hypotonic solution (normal saline solution) maintain their integrity for several hours. Gellan gels can be formed in the tear fluid when the polymer concentration is very low. The sodium ion was found to be suitable for *in situ* formation of the gel.[17]

Gellan was also tested *in vivo* for nasal administration with *in situ* gel formation. S. L. Cao *et al*.[18] have obtained a new gellan gel with *in situ* formation for nasally administrable mometasonefuroate controlled release. This drug (class of corticosteroids) is effective in inhibiting the symptoms associated with allergic rhinitis. *In vivo* tests showed that 20 μg/day mometasonefuroate are enough to reduce the number of sneezes in rats.[19,20] For comparison purposes, the drug was administered to the rats both in suspension and incorporated in a simple gellan solution. It was proved that the effectiveness of the drug from the gel formed *in situ* is greater than the free drug and the effects are improved a lot. The viscosity of the system has a great importance. First, it has the viscosity of a fluid, which can be sprayed, and then the gel if formed in the presence of cations; it must maintain structural integrity to facilitate sustained drug release over an extended period without eroding or rapidly dissolving. Gellan requires small amounts of ions in order to convert into gel; its viscosity increases with an increase in the concentrations of polysaccharide. Xanthan (suspending agent), 0.5% gellan and mometasonefuroate were used in order to formulate the drug transport system. *In vivo* tests proved that the therapeutic effects of the drug can be improved if it is incorporated into the polymer system particularly formulated for *in situ* gelling; the gel formed had pseudoplastic characteristics and was safe throughout the study.[18]

Gellan microparticles containing metoclopramide hydrochloride for intranasal administration were developed by the spray-drying method. The method is not dependent on the solubility characteristics of the drug and the polymer. The microparticles of gellan and metoclopramide are not toxic for the nasal mucosa. The particle size varied between 9.38-10.67 μm, being suitable for nasal administration. After spraying, the microspheres form a gel in the nasal mucosa due to their mucoadhesive properties by withdrawing water from the nasal mucosa and by interacting with cations. Drug release from the microparticles was moderately controlled, being attributed to the formation of the hydrogel. The results of *in vitro* studies are promising and proved that the spray-drying

method used for obtaining gellan microparticles with metoclopramide hydrochloride could be used to develop intranasal systems. Pharmacokinetic and pharmacodynamic studies are required before nasal administration in humans.[21]

Gellan was also tested for oral administration of drugs. Studies were performed to create an *in situ* gelling system for controlled release of naproxen. Naproxen is a NSAID with antipyretic and anti-inflammatory effects. It binds to albumin in the plasma and has gastric toxicity; it would be more efficient if the dose of the drug could be delivered by controlled release over a prolonged period.[22] Three kinds of polymers with gelling properties were used: sodium alginate, pectin and gellan. Calcium chloride and sodium citrate of specific concentration were used as crosslinking agents for each of the polymers in solution. A drug concentration of 2.5% was included in each of the polymer solutions.[23] *In situ* gelation was tested by adding the polymer solution dropwise in simulated gastric fluid at 1.2 pH.[24] The effect of different concentrations of $CaCl_2$ and sodium citrate on gelling properties was observed. The minimum concentrations that maintained the fluidity of solutions prior to administration and caused gelling of the solutions in gastric fluid were 0.25% (w/v) sodium citrate and respectively, 0.075% (w/v) $CaCl_2$. Increasing the concentration of $CaCl_2$ by 0.1% (w/v) (with the same amount of sodium citrate) caused gelling of the formulation prior to contact with simulated gastrointestinal fluids. Also, the release of naproxen in the gel was proved to be affected by the type and the concentration of gelling agents.[23]

The ionic gelling method is well known. The effect of various cations and of the polymer concentration on the encapsulation efficiency of the drug and its release rate at the target site in the gastrointestinal tract was studied. The particles were tested *in vitro* and *in vivo* and showed promising results in controlled release over a period of time, decreasing side effects, increasing the bioavailability and patient compliance due to reduced frequency of administration.[25,26]

A. Verma and J. K. Pandit[27] studied the effect of calcium and polymer concentration on the efficiency of incorporation and drug release. They used floatable gellan particles, in which rifabutin (a drug used to treat resistant infections, such as gastric infection with *Helicobacter pyloris* bacterium) was incorporated. The particles were prepared by ionotropic gelation with calcium ions in an acidic environment. The particles are spherical, have a rough surface and cross-section, highlighting a highly porous interior.[27]

Controlled delivery systems for orally administered drugs are difficult to accomplish because of their limited stationary period in the gastrointestinal tract. A rapid gastrointestinal transit may prevent the release of the total drug dose in the absorption area and therefore the efficiency of the administered treatment decreases. Most drugs are absorbed in the stomach or in the upper part of the small intestine. To overcome these limitations, floating drug delivery systems were prepared. They have a lower density than the gastric fluid and therefore remain floating within the stomach a longer period of time without affecting the gastrointestinal transit; thus, the controlled release of the drug contained within the system is achieved. The materials used for obtaining floating systems for controlled release of drugs include gaseous agents, CO_2. Carbonates and bicarbonates are used as gas-forming agents.[28] The float properties of the system developed by A. Verma and J. K. Pandit[27] depended on the $NAHCO_3$ concentration used. The influence of Ca^{2+} and polymer concentration on drug encapsulation and release was studied. Drug encapsulation into particles varies between 40.3% and 60.7%. Increasing the calcium concentration will increase the efficiency of drug encapsulation.

The use of $NaHCO_3$ as a gas generating agent may release CO_2 when reacting with the acetic acid (from the extrusion bath of particles), causing low efficiency in the immobilization of the drug. Carbon dioxide remains locked in the gel network, thus increasing the porosity of the particles and decreasing the strength of the particle wall. As a result, the drug easily diffuses in the gelling liquid.

Drug release decreases with increasing Ca^{2+} concentration because of particles' increasing crosslinking. There was no difference within the release phase when the polymer concentration was increased. In a first phase, drug release was fast, but later it dropped. *In vitro* tests showed that, in the first hour, 48-68% out of the total drug amount was released.

The study demonstrates that the efficiency of drug incorporation may be controlled by adjusting the process factors. The particles prepared in this way may be used as polymeric particulate systems for the controlled release of rifabutin, which is orally administered for the treatment of various gastric resistant infections.[27]

A. K. Nayak *et al.*[29] developed new gellan particles using tamarind seeds and gellan for the controlled release of orally administered metformin hydrochloride. Tamarind seeds are non-carcinogenic, biocompatible and have an excellent stability at acidic pH.[30] They have also mucoadhesive properties being used in drug delivery.[31] Ionic gelation was chosen as a method for producing the particles. $CaCl_2$ has been used as a crosslinking agent in the formulation of the gellan spherical particles with tamarind seeds. The particles were characterized by SEM and FTIR analyses. The particles thus formulated have a degree of swelling depending on the pH, good mucoadhesivity to biological membranes and the *in vivo* tests on rats have shown good hypoglycemic activity. They may be used for the controlled release of metformin hydrochloride in order to maintain the optimal level of glucose in the blood and good compliance of patients. Metformin hydrochloride is used to lower blood sugar in type 2 diabetes non-insulin dependent. The half-life is 1.5-1.6 h and it is absorbed in the upper bowel.[32,33] The oral bioavailability is 50-60%.[31] The mucoadhesive spherical particles have been optimized by a 3^2 factorial design and analyzed by RSM (response surface methodology). An increase in the efficiency of drug encapsulation with a decreased ratio of tamarind seed–gellan and a decrease in $CaCl_2$ concentration was observed. A decrease in drug release was observed with a decrease of the gellan–tamarind seed ratio and a rising concentration of $CaCl_2$. The encapsulation efficiency is 95.73± 4.02% and the drug release in the first 10 hours is 61.22 ± 3.44%. Such mucoadhesive particles may be used for the encapsulation of other drugs that require controlled release over a longer period of time, to improve their bioavailability and therapeutic efficiency.[29]

The same research group[34] also developed gellan particles with Ispaghula mucilage (*Psyllium*), incorporating metformin hydrochloride. As in the previous study, ionic gelation was used and the crosslinking agent was $CaCl_2$. Mucoadhesive spherical particles have been optimized by a 3^2 factorial design and analyzed by RSM (response surface methodology). The encapsulation efficiency was 94.24 ± 4.18% and the release of the drug within the first 10 hours was 59.13 ± 2.27%. The mucoadhesive particles containing metformin hydrochloride are used for controlled release of drug over an extended period of time. They have antidiabetic properties demonstrated in alloxan induced diabetic rats. After prolonged administration of these particles, it has been observed that optimal blood glucose level is maintained and patient compliance is improved.[34]

Gellan spherical particles prepared by the ionic gelling method have been used for controlled release of cephalexin. The effect of the formulation parameters on the encapsulation efficiency and controlled drug release was observed. Gellan dispersion containing cephalexin was extruded into a bath containing a solution of calcium and zinc ions. The variables for the optimization of the process, such as the pH of the extrusion solution and the amount of cephalexin from the extruded polymer solution, have been modified to achieve effective immobilisation of the drug, controlled release over a prolonged period, and an optimum morphology for the spherical particles. The particles prepared in the acidic environment have a porous structure, while those prepared in a basic medium have a smooth surface. Particle characterization was done by FTIR spectroscopy, laser diffraction and DSC. The particles have spherical shape with an average diameter ranging between 925 and 1183 μm. A 69.24% encapsulation efficiency was obtained. The effectiveness of drug release was the most when a larger amount of drug was immobilized in particles. The particles prepared in acidic environment were released at a higher speed. *In vitro* tests were carried out in 0.1 N HCl or phosphate buffer (7.4 pH), but no significant difference was observed. The study proved that uniform size gellan particles having controlled-release properties of the drug are obtained through combination of calcium ions with zinc.[35]

S. Bhattacharya *et al.*[36] prepared gellan microparticles by the ionic crosslinking technique using aluminium chloride as crosslinking agent, and tranexamic acid was immobilized within the microparticles. The tranexamic acid is an antifibrinolytic drug with a half-life of 1.9-2.7 h and bioavailability of 39%; therefore, it requires repeated administration in order to maintain the optimal therapeutic level. The characterization was performed by scanning electron microscopy, FTIR spectroscopy, X-ray diffraction, DSC and HPLC. The particles are spherical and have a size of 8.11×10^2 μm to 9.11×10^2 μm and their diameter increases with increased concentration of the polymer. The microparticles have a low degree of swelling in acidic environment, but it increases

considerably in alkaline environment. The efficiency of drug encapsulation decreases from 89.12% to 71.15% by varying the concentration of gellan solution from 0.75% to 1.25%. No polymer–drug interactions were highlighted by FTIR spectroscopy. The higher the degree of polymer swelling, the higher the speed of drug release from the microparticles will become. The full release of the drug has been reached in alkaline environment in different periods of time and depended on several factors, such as polymer concentration and crosslinker concentration (Fig. 1).

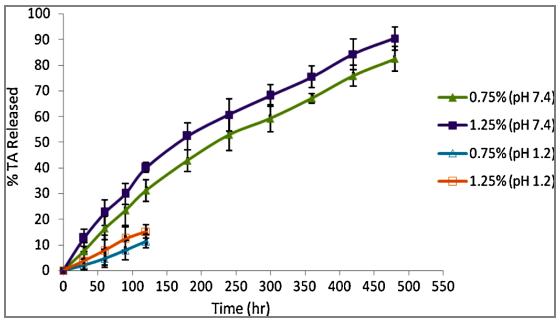

Figure 1: Release profile of TA in acid and alkaline medium from TA loaded GG microbeads prepared for two concentrations of the polymer (0.75% and 1.25%) at a constant $AlCl_3$ concentration of 3% (w/v); (Mean, ±SD, n = 3);[36] (Reprinted with permission from Elsevier: S. S. Bhattacharya, S. Banerjee, P. Chowdhury, A. Ghosh, R. R. Hegde *et al.*, Tranexamic acid loaded gellan gum-based polymeric microbeads for controlled release: In vitro and in vivo assessment, *Colloids and Surfaces B: Biointerfaces*, **112**, 483-491 (2013))

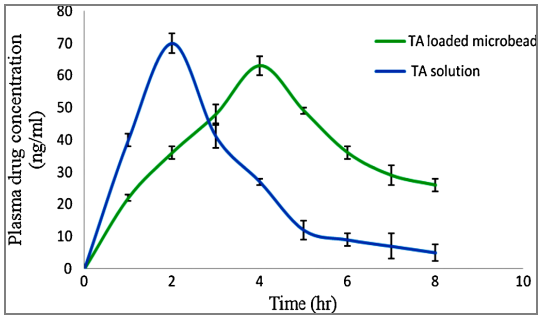

Figure 2: Plasma TA concentration *vs*. time profile in rabbits after single oral administration of TA solution and TA loaded GG microbeads (Mean, ±SD, n = 3);[35] (Reprinted with permission from Elsevier: S. S. Bhattacharya, S. Banerjee, P. Chowdhury, A. Ghosh, R. R. Hegde *et al.*, Tranexamic acid loaded gellan gum-based polymeric microbeads for controlled release: In vitro and in vivo assessment, *Colloids and Surfaces B: Biointerfaces*, **112**, 483-491 (2013))

In vivo tests on rats revealed that the accumulation of drug in blood plasma over time was slower when TA loaded particles were administered, as compared to classic administration. Also, the concentration level was maintained at higher rates, with a duration of up to 8 hours, compared to classical administration. The results obtained are presented in Figure 2.

The results of the study showed that drug-loaded hydrogel microparticles can be used to minimize the release of tranexamic acid in acid environment. Also, they can be used to control the release of a drug in alkaline media, which will help to decrease the loss of drug and increase the bioavailability. *In vivo* tests showed a slow and prolonged release, but more trials are needed.[36]

Aluminium chloride was used as crosslinker for the study of glipizide encapsulation into acetylated gellan particles for controlled and prolonged release of the drug. The method used for manufacturing the particles was ionotropic gelation, followed by covalent crosslinking with glutaraldehyde. Glipizide is a drug that lowers blood sugar and is used in the treatment of type II diabetes.[37] Oral absorption is rapid, uniform and complete with almost 100% bioavailability.[38] The encapsulation in polymer particles may have the effect of increasing its therapeutic effectiveness, given that the half-life of the drug is small (approximately 3.4 hours) and requires repeated administration.[39] A 97.67% encapsulation efficiency was obtained.

The encapsulation efficiency was reduced by 11.89% when glutaraldehyde was used. The rate of drug release was 10% in 2 hours in an acidic environment; under alkaline conditions it was 38-47% for gellan particles ionic reticulated with Al^{3+} and 15% for the particles treated with glutaraldehyde. Drug release was correlated with the degree of swelling in this case, as well as in the study by S. Bhattacharya *et al*.[36] When using glutaraldehyde as chemical crosslinking agent, the rate of drug release decreased. The drug will have controlled release for a prolonged period. These

particles have the potential to be used in controlled release applications and could minimize the frequency of dosing and the side effects associated with the drug.[40]

The complex coacervation method is based on electrostatic interaction between the anionic and cationic polymers, resulting in insoluble spherical capsules.

Chitosan can form polyelectrolyte complexes by electrostatic interactions with polyanions and polysaccharides with COO^- or SO_4^- groups.[41,42] Gellan was tested for the encapsulation of biologically active components in a poly-ionic complex formed with low molecular weight chitosan in aqueous solution. The research led to the conclusion that this polyelectrolyte complex cannot be charged with a large amount of drug because of the acidic nature of the gelling environment, whereas the drug is rapidly released due to the acidic environment of the stomach.[43]

A. Verma *et al*.[44] have chosen a polymeric system, consisting of gellan and chitosan, for piroxicam transport, aiming the controlled drug release in the stomach, to increase the encapsulation efficiency, drug stability and patient compliance, and to avoid the disadvantages associated with gel particles formed in oil-based emulsion. The particles consist of a polyelectrolyte complex based on gellan and chitosan, through the complex coacervation technique, without using any chemical crosslinker. Gelucire (39/01 and 50/13) is used as lipid phase due to its biocompatibility, biodegradability, containing very small amounts of acid and because it prevents stomach irritation by forming a coating around the drug.[45] The diameter of the obtained particles ranges from 1.09-1.3 mm; they show better floatability, increased encapsulation efficiency (the drug is incorporated in the particles in a proportion of 93-98%). Developing floatable particles using a polyelectrolyte complex can have efficient results in the controlled release of anti-inflammatory drugs (NSAIDs) administered orally for an optimal therapeutic effect, reducing side effects (gastric irritation) and providing good patient compliance.[44]

Other studies have been carried out for encapsulating anti-hypertensive drugs. For that purpose, the microspheres were obtained by the method of crosslinking in an emulsion, using gellan and poly(vinyl alcohol) as polymers and glutaric aldehyde as crosslinking agent.[46]

Mechanical strength was enhanced when an interpenetrating polymer network (IPN) was used as compared to microspheres obtained by ionic crosslinking. The obtained particles are spherical. Smaller particles are obtained by increasing crosslinking density due to the formation of a denser polymer network. Increasing gellan concentration leads to a larger size of the microspheres with the formation of several crystalline matrices. The obtained IPN microspheres had a higher tensile strength than that of the microspheres obtained from a single polymer. *In vitro* studies were carried out to determine the release rate of the drug from the microspheres and it was found that the release rate was higher in the microspheres containing smaller amounts of gellan. The efficiency of drug release from the microspheres depends on the environment of diffusion and on the solubility of the drug in different environments.[47]

An interpenetrated polymer network was used in the preparation of gellan and egg albumin microcapsules embedded with a resin-diltiazem complex for the controlled release of drug.[48] Diltiazem hydrochloride, a water-soluble drug, has been linked to Indion 254 – a cation exchange resin. The method used for obtaining gellan-albumin microcapsules was combined with covalent chemical crosslinking ionotropic gelation. The gelling agent used was calcium chloride ion and the chemical crosslinker was glutaraldehyde. Figure 3 schematically represents the interpenetrated matrix formed between gellan and egg albumin.

Microcapsules characterization was done by SEM analysis, DSC, TGA, XRD and FTIR. The microcapsules used were spherical with a rough surface and their diameter varied between 841 and 1118 μm. The particle size decreased with the second crosslinking (with glutaraldehyde) and could be attributed to the rapid contraction of the matrix IPN due to crosslinking covalent bonds between the polymer chains of two polymers. With increased concentration of the glutaraldehyde, the size of the microcapsules decreased due to the increased crosslinking density. When the resinate amount increased, the particles' diameter was enlarged due to the interstitial space filled between the polymer segments. The efficiency of encapsulation was 68.02-89.06%. The encapsulation efficiency decreased with increasing $CaCl_2$ concentration.

The encapsulating efficiency of the microcapsules obtained only through ionic

crosslinking was higher than that of the microcapsules made through double crosslinking. On the other hand, the efficiency of drug encapsulation in the microcapsules prepared with low amounts of glutaraldehyde was lower than when higher amounts of glutaraldehyde were used. Drug release was studied *in vitro* in simulated gastric fluid (pH 1.2) and intestinal fluid (pH 7.4). A simple drug (diltiazem hydrochloride) showed rapid and complete dissolution within 60 minutes. The release of the drug from resinate lasted 3 hours, while from IPN microcapsules it was slower.[47]

Researches were done to obtain gellan gel films with dermatological applications and with controlled release of active principles.

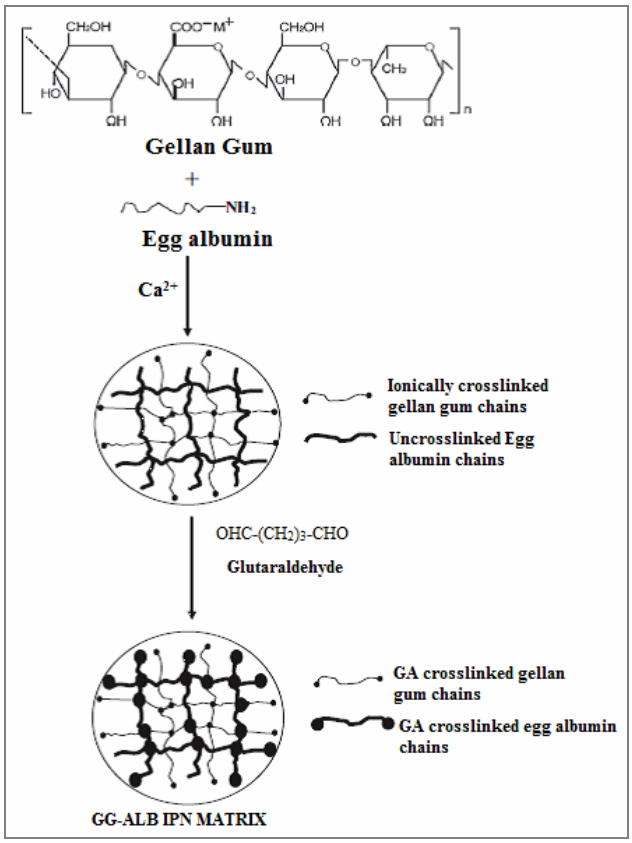

Figure 3: Schematic representation of GG–ALB IPN matrix;[48] (Reprinted with permission from Elsevier: R. V. Kulkarni, B. S. Mangond, S. Mutalik and B. Sa, Interpenetrating polymer network microcapsules of gellan gum and egg albumin entrapped with diltiazem–resin complex for controlled release application, *Carbohydrate Polymers*, **83**(2), 1001-1007 (2011))

Wei Ming *et al.*[49] studied the preparation, characterization and biological properties of gellan crosslinked with 1-ethyl-3-(3-dimethylpropyl) carbodiimide used as a crosslinking reaction activator. In this study, 26 μm thick gellan films were used, which reacted in ethanol (40%) with the crosslinking agent, for medical applications in the treatment of postoperative wounds. Biocompatibility tests were carried out *in vivo* and *in vitro*. The results demonstrated that the gellan film is compatible with the blood cells and fibroblasts L929. The subcutaneous film insertion caused a slight swelling in the first few days after surgery, but

there was no fibrosis or stromal reaction. Such a film has the potential to be used in cutaneous procedures.[49]

C. Cencetti *et al.*[50] prepared and characterized an antimicrobial dressing based on silver, gellan, poly(vinyl alcohol) and borax. Creating a dressing containing silver provides a controlled release of silver and local antimicrobial activity; moreover it allows the dressing to be changed over a longer period of time. This type of dressing should provide a moist environment, avoiding dehydration and adherence to the wound site, as well as keeping the dressing yarn from falling apart. This study describes the preparation of a new (non-woven) dressing on the basis of gellan treated with a mixture of poly(vinyl alcohol) and borax, which is able to increase the encapsulation capacity of silver and to shape its release. The new dressing shows an increase in water absorption capacity (a fundamental property for absorbing wound exudates) and a lower rate of dehydration. Silver is delivered by controlled release over a period of time and its antibacterial activity was tested on *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[50]

C. Cencetti *et al.*[51] also reported a new hydrogel of sulphated hyaluronic acid and gellan gum, which is able to form a barrier against postoperative epidural scars. The new hydrogel contains 2% (w/v) gellan and 1% (w/v) hyaluronic acid sulphate. The presence of sulphate induces hydrophilic properties to the polymer and the intersection with the junction areas of gellan allows obtaining a hydrogel with improved mechanical and rheological properties, and is extruded more easily.[51]

Studies were carried out to obtain a polymer system used in the controlled release of ciprofloxacin with potential dermal application. This polymer system contains gellan derivatives with quaternary ammonium groups. It was obtained by nucleophilic substitution of gellan primary hydroxyl groups of N-(3-chloro-2-hydroxypropyl)-trimethyl ammonium chloride in the presence of alkali, under certain reaction conditions, using different molar ratios of gellan/N (3-chloro-2-hydroxypropyl) trimethyl ammonium-chloride. ^{1}H NMR, FTIR spectroscopy and conductivity titration with $AgNO_3$ were performed to determine the quartenizing degree. Thermogravimetric analysis was used to investigate the thermal behavior.

Particles based on quaternized gellan and chitosan, retaining the antibacterial activity of the quaternary ammonium, have been formulated. *In vitro* studies were performed on rat skin in phosphate buffer (pH = 7.43). Ciprofloxacin was released in 24 hours (Fig. 4), confirming that the particles thus obtained can be used as systems for the controlled release of drugs with local dermal applications.[52]

MEDICAL APPLICATIONS

In recent years, cell transplantation therapy has played an increasingly important role in regenerative medicine. The quantity and quality of cells play a decisive role for an adequate treatment. Polymeric supports are used as vehicles for the transport and controlled release of therapeutic cells to a target place in the body. They also serve to maintain cell viability and cell phenotype that need to remain intact during transport.[53,54]

Studies have been conducted to obtain new polymeric systems. These systems are applicable in tissue engineering and regenerative medicine and should be able to carry and deliver cells that treat various diseases. C. Wang *et al.*[55] have obtained gellan-based hydrogel polymer supports that help regenerate various tissues and are used as transport and controlled release vehicles for cells. Gellan microspheres are prepared by the water-in-oil emulsion method, followed by a series of redox crosslinking treatments. To improve cell adhesion, a layer of gelatine is used to cover the surface of the microspheres, resulting in a gelatine grafted–gellan support used in cell transportation. The cells that are immobilized on the support are actually human dermal fibroblasts and human fetal osteoblasts. Using optical microscopy and scanning electron microscopy, it has been observed that fibroblast cells are spread equally and quickly populate over the gelatine-gellan microsupport surface (Figs. 5, 6).

In the case of osteoblastic cells, which are immobilized on the same type of support, cell viability and potential for osteogenesis are demonstrated by fluorescence assays, histological and biochemical specific indications. The gellan and gelatine microspheres may be used in cell transportation with applications in tissue engineering and regenerative medicine.[55]

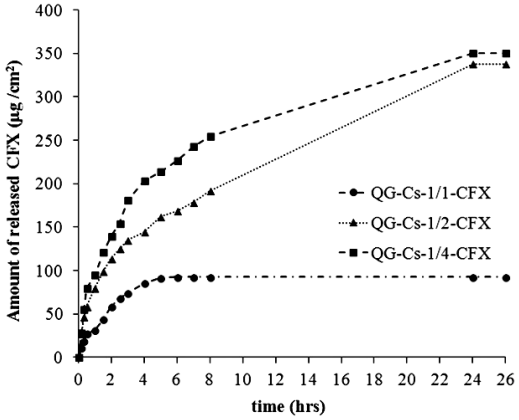

Figure 4: *In vitro* permeation profiles of CFX from QG–Cs particles through rat skin;[52] (Reprinted with permission from Elsevier: O. Novac, G. Lisa, L. Profire, C. Tuchilus and M. I. Popa, Antibacterial quaternized gellan gum based particles for controlled release of ciprofloxacin with potential dermal applications, *Materials Science and Engineering*: C, **35**, 291-299 (2014))

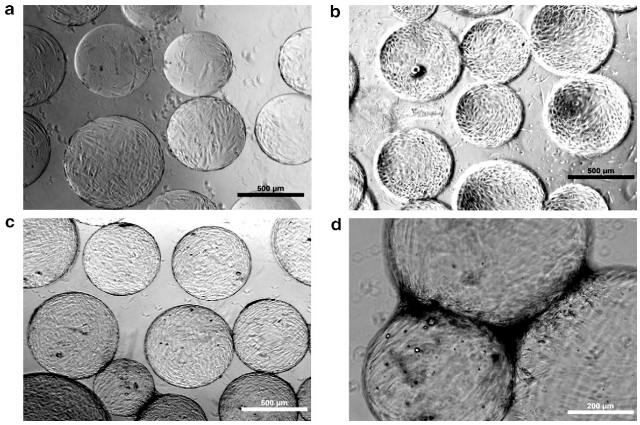

Figure 5: Optical microscopic morphology of HDFs cultivated on TriG microspheres: (a) day 3, 40; (b) day 7, 40; and (c) day 14, 40 (scale bar = 500 µm). The cell growth-induced inter-microspherical conglutination is highlighted with greater magnification (d, day 14, 100, scale bar = 200 µm);[55] (Reprinted with permission from Elsevier: C. Wang, Y. Gong, Y. Lin, J. Shen and D. Wang, A novel gellan gel-based microcarrier for anchorage-dependent cell delivery, *Acta Biomaterialia*, **4**(5), 1226-1234 (2008))

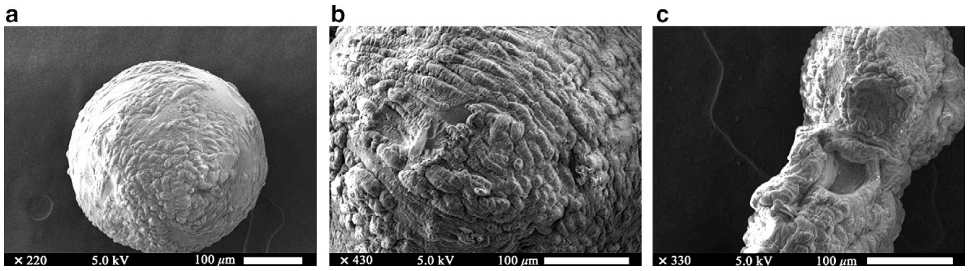

Figure 6: FESEM observation of HDFs cultivated on TriG microcarriers on day 7 (a), whose detailed adhering morphology is highlighted in (b). The cell growth-induced inter-microspherical conglutination is highlighted in (c) (scale bar = 100 µm);[55] (Reprinted with permission from Elsevier: C. Wang, Y. Gong, Y. Lin, J. Shen and D. Wang, A novel gellan gel-based microcarrier for anchorage-dependent cell delivery, *Acta Biomaterialia*, **4**(5), 1226-1234 (2008))

Articular cartilage is considered to be the most important cartilage in tissue engineering applications, due to its function in mobility and locomotion. Injuries and degenerative diseases (osteoarthritis, rheumatoid arthritis) can cause disability states, leading to pain and decreased quality of life. Several treatments have been tested, but the results were not satisfying and therefore more studies have been done in this area to develop other techniques for treating these disorders.[56,57]

Biopolymeric substrates were studied to incorporate cells and to form a functional cartilaginous tissue.[58] Injectable hydrogel systems can be applied by minimally invasive techniques. They have been used in cartilage regeneration due to their gelation ability *in situ* under physiological conditions.[59]

Gellan was one of the biopolymers proposed for tissue engineering applications.

J. T. Oliveira *et al.*[60] studied an injectable hydrogel-based gellan containing autologous cells used for cartilage regeneration of affected rabbits. The cells encapsulated in the gellan hydrogel were human articular chondrocytes. The hydrogel and the cells were placed by injection into the affected articular cartilage and the gelation process took place *in situ* under physiological conditions. After 8 weeks of action, histological, biochemical, molecular and immunological tests were performed. The results showed uniform cell distribution. Also, the chondrocytes in the matrix proved to be round-shaped.[60]

J. Fan *et al.*[61] also used gellan hydrogels for injection with built-synovial mesenchymal stem cells (CSMS) taken from rabbits. The system was tested *in vitro* for cartilage tissue engineering applications. Rabbit CSMS chondrogenesis with gellan injectable hydrogel was tested *in vitro* using chondrogenic cultures. The results showed the formation of cartilage by treating cell cultures with TGF-β1, TGF-β2 and BMP-2, which led to the conclusion that CSMS incorporated gellan injectable hydrogel has great potential for cartilage construction. These studies are the basis for future *in vivo* investigations.[61]

However, the high temperature and lack of strength of the gelling agent limit the use of gellan in tissue engineering. Research has been conducted to improve these properties. A new biomaterial based on gellan-chitosan has been obtained with possible applications in tissue engineering. Gellan was first oxidized and then mixed with carboxymethyl chitosan, which led to a complex hydrogel consisting of a double network. There was an improvement in gelling temperature of less than 42 °C and an increase in compression modulus of 278 kPa. Also, after compression, the hydrogels can return to their original shape. Chondrocytes were encapsulated into this new hydrogel, and *in vitro* tests have shown that they proliferate and have an increased viability.

The hydrogel based on gellan and carboxymethyl chitosan is a material that may be used in tissue engineering applications for cartilages.[62] Gellan can be modified using meta-acrylic techniques. In this case, an alternative method for obtaining hydrogel photopolymerization can be used. The use of hydrogels photo-polymerization leads to improved mechanical and structural characteristics.[63]

Obtaining hydrogels with high mechanical strength is a major objective in tissue engineering applications. To achieve this objective, one strategy would be to create a double network hydrogel that can encapsulate cells. Studies have been carried out in this direction and a double network hydrogel formed by photo-crosslinking was developed in two steps. The first rigid and fragile network used gellan methacrylate polymer and the second soft and elastic network used methacrylamide/gelatine. The mechanical properties of the each network were obtained by controlling the degree of the meta-acrylic process of each polymer. The dual network is formed by photo-crosslinking of methacrylate gellan, the diffusion of methacrylamide gelatine in the first network, and the photo-crosslinking of methacrylamide gelatine to form the second network. The compressive strength of the hydrogels produced by this method was up to 6.9 MPa, a resistance value that is close to the one of the cartilage. The mass ratio of polymer hydrogels influences the mechanical resistance. Cells and fibroblast cells have been encapsulated in these networks. The tests showed that the compatibility and the cells viability were not affected by the processing conditions of the hydrogel.[64]

In terms of angiogenic potential, tests were performed to investigate the application the gellan hydrogel in nucleus pulposus regeneration. Hydrogels for regeneration of nucleus pulposus must have non-angiogenic or anti-angiogenic properties. Because of the ability of gellan

hydrogels to achieve supports for encapsulating cells with optimum mechanical properties and the lack of toxicity, they can be used as substitutes for the nucleus pulposus in various cell-free or cellular strategies.

The chorioallantoic membrane test was performed to determine whether gellan hydrogels have angiogenic response. For that purpose, gellan hydrogel discs, respectively ionic cross-linked methacrylate gellan, were used. This test demonstrates that gellan hydrogels have no angiogenic response, do not inhibit nor induce the formation of new blood vessels. Histological and immunohistochemical tests have shown that the hydrogels are non-permissive to the restoration of endothelial cells. The ionic crosslinked or photo-crosslinked methacrylate gellan hydrogel does not cause any acute inflammatory response. Angiogenesis is a process that is strongly associated with the progressive deterioration of nucleus pulposus, which could be prevented by using methacrylate or ionic photo-crosslinked gellan hydrogel, because the hydrogel can help regenerate the damaged tissue of the intervertebral disc. Due to their functional and adjustable properties and their ability to control the infiltration of cells and the invasion of blood vessels, gellan hydrogels have the potential to be used as nucleus pulposus substitutes.[65]

Studies were conducted to achieve regeneration and repair of osteochondral defects. These defects occur after a trauma, cancer or metabolic dysfunction. Osteochondral defects require regeneration of cartilage and subchondral bone at the same time, which involves certain bi-layered construction.[66] Bilayered constructions represented by a bone and a cartilage structure were proposed by J. M Oliveira.[67] The systems associate biphasic and biomimetic constructions, and are composed of hydroxyapatite and chitosan.[67]

Observations revealed that hydrogels are able to treat osteochondral defects. Gellan forms hydrogels that can gelate *in situ*. Due to their characteristic structure, these hydrogels are used in the regeneration of cartilage tissue. In recent research done by Diana R. Pereira *et al*.,[68] low acyl gellan was used for bilayer hydrogel constructs in osteochondral tissue engineering. Bilayered constructions have been obtained from an aqueous solution of 2% low-acylated gellan (cartilaginous structure) and a solution of 2% low-acylated gellan containing different amounts of hydroxyapatite (5-20%), thus the bone structure was achieved. The bioactivity was assessed *in vitro* by immersing the construction of the hydrogel into a solution of simulating body fluid for 14 days. The characterisation of the hydrogel obtained by scanning electron microscopy, FT-IR and X-ray diffraction showed that the formation of the apatite layer was limited by the bone structure. Bioactivity tests show a different behaviour in both structures. The bone structure is bioactive (the apatite layer was formed in contact with the simulated body fluid), while the cartilaginous structure showed no bioactive nature. The presence of hydroxyapatite induces apatite nucleation and calcium ions accelerate this nucleation in the bone-structure layer. Gellan bilayered hydrogel constructs have a major potential that can be used in tissue engineering.[68]

Limited regeneration of articular cartilage has encouraged further research to develop new treatments and investigating new medical techniques to achieve this objective. The incorporation of the stem cells, precursor cells along with growth factors in various media based on polysaccharide hydrogels represents an approach to articular cartilage regeneration.[69] The substrate rigidity can influence the cells behaviour and can support cell differentiation in different phenotypes of cells.[70-72] Soft substrates tend to promote neurogenic, adipogenic and chondrogenic differentiation, while the more rigid substrates support myogenesis and bone formation depending on the specific composition of the culture media.[73,74]

M. Ahearne and D. J. Kelly[75] compared fibrin hydrogels, agarose and gellan as microspheres for transportation and incorporation of stem cells and as growth factors used in applications for regeneration of cartilage. MSs gelatine has been used to control TGF-β3 growth factor. The precursor cells (progenitor) extracted from the infrapatellar fat of the knee was used to determine the ability of the three hydrogels to support hyaline cartilage formation. The studies were performed *in vitro*. The highest level of DNA was observed from gellan hydrogels containing TGF-β3 growth factor and release MSs. The gellan use is limited by rapid gelation of the hydrogel at low temperatures; therefore it is necessary to optimize the ratio of the gellan and $CaCl_2$ concentration in order to obtain a hydrogel with a suitable gelling temperature.[75]

Injured adult tissue of the central nervous system has very limited regeneration ability. Spinal cord injuries can lead to permanent loss of

motor and sensory functions, as well as to other complications. Drug approaches are limited and are based on anti-inflammatory drugs, such as methylprednisolone.[76] Stem cell transplantation may be a strategy for regeneration of the tissues, but the cells have a low rate of survival (under 1%). To increase the viability of these cells, they can be immobilized in a biopolymer matrix that can provide them with a survival environment after transplant. Gellan is used as biomaterial for cell transport, but to increase cell adhesion it should be modified with a systemic peptide derived from fibronectin. Modified gellan has a profound effect on morphology and neural stem cell proliferation, different from the effect of unmodified gellan on the same type of cells. This study demonstrates that the modified gellan used as ~~a~~ biomaterial could have benefits in the treatment of spinal cord injuries.[77]

APPLICATIONS IN THE COSMETIC INDUSTRY

In the cosmetic industry, gellan is used more as a structuring agent than as a gelling agent for sunscreen products, lotions, hair conditioners *etc*. It provides product stability and a pleasant sensation when applied on the skin. Product properties are improved by adding hydrocolloids, such as xanthan. Deacetylated gellan and high acyl gellan were tested to determine their purity and to establish consumer compliance for these products.[78,79] The incorporation of gellan in hair care products, shampoos and conditioners provides a greater stability, better structure and pseudoplastic properties to the products. The concentration of gellan in shampoos or conditioners is 0.2% or less when using other polymers in the formulation of these products.

Creams and lotions are emulsions in water. They should not contain any trace of oil. By using gellan, these emulsions become more stable, even at higher temperatures, and offer a bright and silky skin feel when used. The concentration used is up to 0.25%. Body lotions containing gellan have the ability to carry important ingredients evenly on the skin. It is also a very effective ingredient in sunscreen creams and lotions.[80]

Gellan is used in the formulation of toothpastes due to the binding properties and texture that the gel provides to products. It has the ability to release flavour, and this leads to a reduction of the concentration of flavour and saccharin in the product up to 10-25%. Reversible gel structure is created, thus leading to reduction or complete elimination of silica thickening used in some formulas for toothpaste production. Gellan is used in low concentrations and contributes very little to the viscosity of toothpaste; during the preparation, the mixture has low viscosity and is easy to pack. After packaging, a gel is formed.[81]

Toothpastes containing gellan have an easier formulation and packaging, and allow the incorporation of more sensitive ingredients (active ingredients, flavours), which would not be possible with typical binders. In toothpaste production, other hydrocolloids, such as xanthan, carboxymethyl cellulose, hydroxyethyl cellulose, carbomer and carrageenan, may be used to provide a better taste and superior properties of binding with water. Higher concentrations of gellan allow other hydrocolloids to provide a smoother consistency to the mixture.

The advantages of gellan in toothpastes are the following: low concentrations (0.025-0.25%); reversible gel structure; provides stability to temperature (gel structure resistance up to 60 °C); contributes to the low viscosity of the mixture for the preparation of the toothpaste; gel structure occurs after several hours of preparation; it has a great capacity to release flavours, which allows the use of lower concentrations of flavours and sweeteners; toothpastes based on gellan have stable enzymes, pH and shear; it is compatible with active agents, such as anti-plaque agents.[80]

OTHER APPLICATIONS

Gellan can be used in biotechnology as a microbiological growth medium, as an alternative to agar.[82] It is also a proper environment for the cultivation of plant tissue.[83] Growing tissues and morphogenesis may vary for different plants according to their nutritional requirements. Tissues in different parts of the plant may have different food needs for optimal growth.[84]

The growth medium for plant tissue contains the following components: macronutrients, micronutrients, vitamins, amino acids or nitrogen supplements, carbon sources, undefined organic supplements, growth regulators and hardening agents. The International Association for Plant Physiology defines macroelements as compounds in culture medium that are at a concentration higher than 0.5 mM/L, while those with a concentration lower than 0.5 mM/L are named micronutrients. The growth rate varies between plant species and therefore the optimal concentration of each nutrient that is found in the

culture medium must be taken into account.[85] The hardness of the culture medium influences the plant tissue growth.[86]

Gellan gels are heat stable and can withstand prolonged incubation at elevated temperatures, at lower concentrations than agar. Based on these properties, gellan is used as a culture medium for thermophilic microorganisms.[87]

Gellan used as microbiological medium is called Gelzan™CM and is sold by Kelco. They have conducted studies on 50 different bacteria, which proved that gellan can not only replace agar in a variety of conventional microbiological applications, but also can contribute to better cell proliferation in some cases.[82,88,89]

In crops, gellan is a promising alternative to agar due to its purity and clarity.[90] The concentration used is five times lower than the concentration of agarose, it is contamination resistant, easy to clean and allows clear observation of roots and plant tissue development in the culture medium.[91]

Various studies have been conducted on the use of gellan as a medium for plant tissue culture. Other studies aimed assessing nitrogen fixation by diazotrofic bacteria determined by measurements of acetylene reduction in nitrogen-poor environments, using gellan as a medium.[92] A solidified soft gel containing 0.5% sugar and 0.2% agar is most often used for acetylene reduction tests and for evaluating nitrogen fixation by various aerobic bacteria, such as diazotrofic bacteria culture medium.[93,94] Gellan microbiological medium at 0.3% concentration may be used instead of ordinary agar culture medium.[91] The advantageous physical properties of gellan allow free live bacteria cultures that fix nitrogen.[95] In contrast to the microbiological medium of agar, gellan medium can prevent DNA extraction or PCR sequences isolated from colonies of microorganisms that grow in the gelling medium.[96,97] Some soil bacteria, which are difficult to grow or grow poorly, are cultivated in gellan medium and colonies grow until they become visible.[98] Gellan supports the growth of test bacteria isolated in the culture medium, and this has led to the idea that an increase in acetylene reduction of nitrogen-fixing bacteria is due to the gellan-based microbiological environment.[92]

The cleaning procedure is an important step in preserving paper. Substances that degrade paper are removed by washing them with water treatment. This method has some disadvantages, such as frequent replacement of water, prolonged contact with water leads to swelling of cellulose fibers (which causes deformation of the paper after drying); modern paper cannot be cleaned by this technique because it is fragile and sensitive to water.[99-101]

Because of the disadvantages of conventional cleaning treatment, new cleaning techniques using suitable hydrogels have been developed. The penetration of liquids into paper, and therefore the swelling and deformation of paper, can be reduced significantly by the use of hydrogels. Optical qualities of paper-based artefacts can be improved and dust from cellulose degradation may be removed.[102,103] Hydrogels used in treatments to clean old paper artwork must be rigid and must form films, so that they can be easily removed by a single operation. Gel residues must not remain on paper because they can cause dangerous microbial growth on the surface.[100,102,104]

Deacetylated gellan forms a hydrogel with an optimal strength, rigid and low syneresis in the presence of bivalent ions. It is also clear, homogenous and resistant to temperature and pH.[105] Due to its pH stability and taking account the other functional properties of gellan hydrogel, it was chosen for new cleaning paper treatments; cleaning can be applied to works of art manufactured from this material. C. Mazzuca *et al*.[106] compared and discussed the results achieved by applying an innovative gellan hydrogel and traditional cleaners with water, using paper samples dating from different periods (16^{th}-17^{th} centuries). Tests were carried out to determine the state of degradation of paper samples using various techniques such as high performance liquid chromatography (HPLC), FTIR spectroscopy, scanning electron microscopy (SEM) and calorimetric analysis. The tests demonstrated the effectiveness of the cleaning method using the gellan hydrogel.[106]

CONCLUSION

The biocompatible character of gellan determines its use in various applications where this polysaccharide works in contact with or inside the body. Due to its capacity to be formulated as particles, films and more or less fluid hydrogels, gellan is an ideal vehicle for orally administrable drugs, transdermal, airway, instilled into the conjunctival sac of the eye *etc*., and it has the ability to cause sustained controlled release of active principles. All these properties

determined numerous applications in medicine, pharmaceutical formulations, in cosmetics or tissue engineering. Besides these fields, gellan is used also in various applications in the food industry or in biotechnology (immobilization of enzymes, yeast cells *etc*.).

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