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Pharmaceutical applications of lectins

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ABSTRACT

Oral delivery of drugs is the most convenient administration route. Since the Gastrointestinal Tract (GIT) represents a hostile environment for most of drugs, development of more sophisticated formulations are required to enhance drug absorption and reduce their adverse effects. Over the last few years, targeted drug delivery systems have been the object of intense investigation. Lectin-mediated drug targeting has been positioned as one of the most promising approaches for drug targeting because of its great specificity. Both direct and reverse lectin targeting studies has been analyzed, specifically those focusing on drug targeting to the intestine. This strategy is not only useful in intestinal diseases, but also provides an increase in the systemic absorption of drugs.

Contents

1. Introduction

Many of the biological roles of glycans are mediated via recognition by Glycan Binding Proteins (GBPs). In nature, we can find two main types of GBPs: lectins and glycosaminglican binding proteins. In this article, we will focus on lectins [1].

Lectins are proteins that recognize and bind to sugar complexes attached to proteins and lipids with very high specificity. This binding is possible because they have a carbohydrate recognition domain (CRD) within its polypeptide structure. A representative characteristic of lectins is their ability for agglutinating erythrocytes. Because of this capacity they are also known as agglutinins [2,3]. They are widely used in drug targeting research as they provide appropriate specificity and show resistance to enzymatic degradation.

Lectins were described by the first time by Doctor Hermann

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Stillmark in 1888, when he described the agglutinating properties of ricin, a toxic lectin extracted from castor seeds. In 1972, Sharon and Lis listed different lectins known at that moment, starting the modern era of lectinology. Since then, the number has increased dramatically [4].

The most common classification for these proteins is based on their source. They are prevalent in animals and plants, as well as in the microbial world, wherein they usually tend to be called by other names such as hemagglutinins and adhesins [5]. During the Second World War, the interest in blood typing for blood transfusion resulted in the discovery of some lectins specific for various blood types. These agglutinins were thus renamed "lectins," a term derived from the Latin word "legere," meaning "to select." Lectins have been found in almost every plant species studied and are particularly abundant in the seeds of leguminous plants. Viral lectins are usually called hemagglutinins and bacterial lectins fall into two classes: lectins (adhesins) that reside on the bacterial surface and facilitate bacterial adhesion and colonization and secreted bacterial toxins. Animal lectins were first described in the body fluids of diverse crustaceans and arachnids, and afterwards they were detected in vertebrates [1,6].

It is also possible to classify lectins attending to their sequence and structural homology (Fig. 1). Lectins that require calcium for recognition were named **C-type** lectins. S-type lectins were a group that required free thiols for stability (nowadays known as **galectins**, as not all of them were thiol-dependent). The lectins that recognize Man-6-P were designated as **P-type** lectins. In the 1990s was discovered that immunoglobulins could distinguish carbohydrates, leading to a new group of **I-type** lectins. A subgroup of these molecules, which specifically bind to sialic acids, has been designated as **Siglecs**. At the present time, there is no single universally accepted classification of lectins.

In use of lectins towards glycotargeting, Drug Delivery Systems (DDSs) are decorated with lectins of certain carbohydrate specificity so that it can interact with glycosilated surfaces [7]. Different cell types express different glycan arrays and in particular diseased cells often express different glycans compared to their normal counterparts. Therefore, lectins could be used as carrier molecules to target drugs specifically to different diseased or normal cells and tissues. Their structure and sugar specificity determine if they will be endocytosed by target cells or not. Thus, the selection of a suitable lectin may allow the cellular uptake and subsequent intracellular routing of such delivery systems to be controlled [8]. This strategy is expected to improve absorption and bioavailability of poorly absorbable drugs, peptides and proteins as well as therapeutic DNA [7].

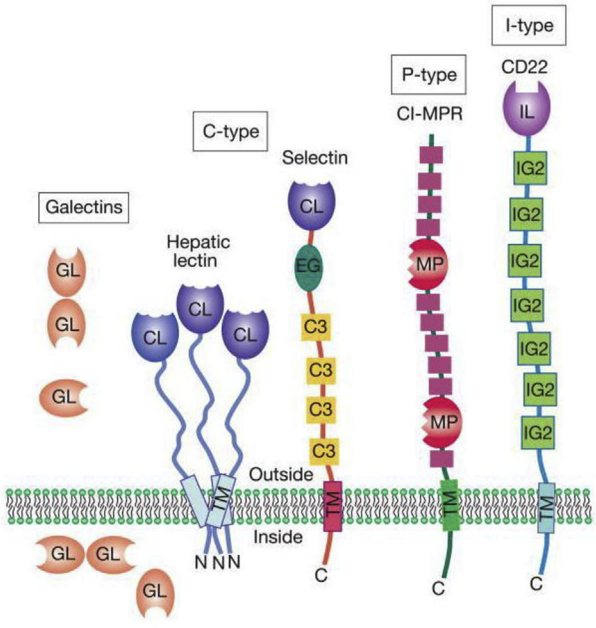

Fig. 1. Lectin classification attending to their sequence and structural homology.

There exist two strategies of using lectins for drug targeting, relying on use of either the oligosaccharide moiety or the lectin as a component of the drug delivery system [8]. **Direct lectin targeting** or glycotargeting consists in DDS possessing carbohydrates (oligosaccharides or neoglycoconjugates) that are recognized and internalized by cell surface endogenous lectins. In vertebrates we can find C-type lectins (selectins, pentraxins), which require calcium for carbonate binding, and S-type lectins or galectins, which are calcium independent [8–12]. For example, galectins 1 and 3 are expressed on normal colon cells and overexpressed in colon cancer cells [13,14]. In contrast, galectin 8 is less expressed in colon cancer than in normal colon cells [15]. **Reverse lectin targeting** approach utilizes exogenous lectins as targeting moieties that target whole DDS to glycoproteins or glycolipids expressed on the surface of intestinal cells. Plant lectins are mainly used for this purpose [16,17].

Then, the main objective of this review is highlighting the principal applications of these proteins in pharmaceutical development as excipient in drug targeting, or to produce useful in vivo model in pharmacokinetics assays.

2. RIL or RIP-based immunotoxins

Ribosome inactivating lectins (RILs) is a specific group of lectins isolated usually from plants. RILs or type 2 ribosome inactivating proteins (RIPs) consists of a polypeptide lectin chain (B chain) connected by a disulfide bridge with a ribosome inactivating chain (A chain). In one hand, the A chain exhibit specific and irreversible ribosomal RNA N-β-glycosidase activity, inhibiting protein synthesis in mammalian cells. For that reason, it should be considered as an interesting toxic part in the design of immunotoxins. On the other hand, from a pharmacotechnical point of view it should be considered that toxicity of RILs depends on the following factors [18]:

a) A chain should be able to reach ribosomes. Physiologically, due to the affinity of the lectin chain to galactose residues of glycoproteins or glycolipids on the surface of mammalian cells, RILs are able to overcome cellular barriers. After binding, RILs are endocytosed and transported through the Golgi network by retrograde transport to the endoplasmic reticulum (ER). Once in the ER, the A chain is enzymatically released and partially unfolded and in this way, is able to access to the cytosol, where is refolded again. So deficiencies on the activity of thioredoxin reductases and disulfide isomerases at this level, could jeopardize the toxicity of the A chain. Once in the ribosome, the A chain is ready to depurinate the rRNA in a highly conserved alpha-sarcin loop and in this way blocks the synthesis of proteins and kill the cell. It should be taken account that RIPs in general are produced by plants as a mechanism to defend against predators [19].
b) The enzymatic domain of the A chain is highly conserved and its activity and specificity should be related to intrinsic structural conformation due to for instance to physiological changes in the pH.
c) The lectin activity of the B chain depends on the ability of the residues on the extreme ends to bind galactosides on the surface

of plasma membranes. In the frame of this assumption, RILs should by divided into two categories, toxic RILs (*i.e.* ricin) and "non-toxic" RILs (*i.e.* ebulin). On other words, high or low efficient galactose-binding lectins related to little changes in the arrangement of specific residues. Although IC_{50} values on cultured animal cells and LD_{50} results on mice values are quite different, toxic and "non-toxic" RILs display similar N-glycosidase activity on ribosomes [20]. This could be related to differences not only on binding ability to galactosides on surface of membranes, but also to different intracellular fate as recently have been hypothesized by Girbes and coworkers. Consequently, "non-toxic" RILs are interesting candidates in the design of immunotoxins due to absence of unspecific toxicity related to toxic RILs.

Immunotoxins are prepared coupling a targeting unit, habitually and antibody or related, with a toxic unit [21,22]. Type 1 RIPs (only A chain RIPs) or A-chain excised from a type 2 RIL have been used in the design of these drugs (Table 1). Some of this targeted-A-chain RILs are being studied in different clinical trials. For instance, anti-B4-bR immunotoxins from ricin as toxic moiety is in phase 3. In this sense, our research group has recently developed a new anti-endoglin immunotoxin containing recombinant musarmin 1 (type 1 RIP) linked to the mouse anti-human CD105 44G4 mouse mAB, that specifically killed L929 transfected fibroblast mouse cells with IC_{50} values in the range of 5×10^{-10} to 10^{-9} M [23].

The main drawbacks related to the original design of RIPs immunotoxins are low stability of the chemical bridge between units, low technological performance, immunogenicity or low efficacy due to erratically penetration in tumour, among others. In order to improve efficacy and reduce side effects, fragments of monoclonal antibodies like anti-CD markers, have substituted classical antibodies. Besides, murine antibodies have been humanized with the replacement of mouse domains with human domains. Some authors have also linked single chain variable fragments in order to improve tumour penetration and recovery during manufacturing [38].

Enzymatic chain of RILs/RIPs should be also conjugated to antitumor specific ligands, cytokines among other targeting moieties. As an example ebulin l-transferrin conjugate decreased the IC_{50} of the proteins from 1.5×10^{-8} M to 3.5×10^{-10} M in HeLa cells [39].

RIL- or RIP-based platforms have been recently designed to control the delivery and release of specific drugs. In this sense, antitransferrin-Ab curcin-folate conjugated gold nanoparticles has been used for drug targeting toward brain cancer [40].

3. *Sambucus* RILs model for mouse gut injury and regeneration

Inflammatory bowel disease (IBD) is an idiopathic chronic disease that has emerged in the last years. Among others theories it has been hypothesized that cleanliness and the related loss of intestinal worms may have played a part. More than sixty animal models have been developed to study IBD. Although some cultured animal cells test are available in the first screening studies, animal models are essential for in vivo mechanistic studies and preclinical evaluation of new drugs. Mouse models for the study of experimental IBD could be classified in four categories [41]:

a) Chemical induction: oxazolone, DSS, DNBS, TNBS, PG-PS or acetic acid have been used. These models are easy to induce and are inexpensive but all of them have diminished pathogenic relevance to the human condition.
b) Cell transfer: (i.e. CD45RB model) that performs chronic features of intestinal inflammation but use of immunodeficient mice.
c) Genetically engineered mouse (conditional or conventional knock-out mouse or conventional transgenic mouse): these models are useful to determine the role of specific genetic mutations or target specific cell types but have low pathogenic relevance due to lack of single gene deletion in the human disease.
d) Congenic (SAMP1/YitFc mice): in one hand these models are multifactorial disease with increased pathogenic relevance to the human condition but on the other hand are quite expensive and have poor breeding ability.

Since RILs triggers important physiological inflammatory intestinal alterations, it should be used also to generate animal models [42]. Administration of intravenous sub-lethal concentration of nigrin b to mice promoted a specific and reversible effect on crypts, firstly in the small intestine and afterwards the large intestine and stomach [43]. A more detailed histological research about this model has revealed that intestinal stem cell niche was not totally eliminated, thus allowing the rapid cycling of the stem cells to support crypt regeneration through functional crosstalk with other cell types such as Paneth cells, pericryptal myofibroblasts, and epithelial cells [44–46].

This should be taken into account for its potential use in pharmacokinetic characterization of drugs and nutrients [47].

Table 1
Main RILs used for the design of immunotoxins [24–37].

RIL	Type
Abrin-a	2 (A-chain)
Dianthin-30	1
Gelonin	1
Momordin-I	1
PAP-II	1
Saporin-6	1
Ebulin-l	2 (A-chain)
Nigrin-b	2 (A-chain)
Ricin-a	2 (A-chain

4. Lectin-mediated delivery to the gastrointestinal tract

The gastrointestinal route is the most convenient method for delivery of drugs and vaccines because of its lower costs and the patient compliance, and the supply can be made either orally or rectally. The administration of drugs to the gastrointestinal tract (GIT) usually involves immediate release formulations such as tablets or capsules, as well as extended release ones. Although these formulations are still preferred for their relative simplicity, in an increasing number of cases more sophisticated formulations are required to develop the best possible product [48].

From a technological point of view, GIT is one of the most challenging routes of administration. In the last years, numerous formulations have been developed incorporating nucleic acids, peptides, proteins and other bioactive molecules able to modulate a series of pathophysiological processes. It is necessary to overcome diverse physical-chemical barriers that hinder the administration of active molecules orally and may result in a poor drug bioavailability. The main obstacles are enzymatic degradation, peristalsis, stability at acid pH or resistance to penetration on the surface of the mucosa [49]. These conditions along the GIT, which suffer wide variations, present opportunities to effective drug delivery. All of this makes more challenging and more essential the formulation of new oral drug delivery systems (DDSs) able to overcome these problems [50].

Among the barriers encountered with the GIT, the mucus gel layer covering its surface has major impact on the efficacy of DDSs since it makes interaction with active agents difficult. This layer covers a wide pH range (from 1–2 in the stomach to 7–8 in the colon) [51] and protects the underlying epithelium from toxic substances by its continuous secretion and by forming polyvalent adhesive interactions. It presents sialic and sulfonic acid substructures that supply a net negative charge [52].

Another of the most important factors that limits the oral absorption of drugs is the intestinal residence time. An increase in the residence time is sought in order to favor the contact with the absorption surface and, consequently, improve the drug bioavailability [52].

In addition, the cell surface is formed by intimately compacted microvilli and expresses carbohydrates anchored to the membrane with adsorbed enzymes that create an extremely hostile and degradative environment. The villi of the small intestine house a dynamic population of epithelial cells that includes absorptive, secretory and endocrine cells. The crypts replace the epithelial layer of the intestine every 3–5 days. Absorption takes place in the enterocytes and its neighboring cells [53].

The less hydrolytic section of the GIT is the colon, where additionally exist specific transporters. Furthermore, it is a highly responsive site for the absorption of poorly absorbable drugs. For these reasons, delivery of drugs via the colon is a significant alternative which is gaining importance especially in the treatment of colon diseases such as ulcerative colitis, colorectal cancer and Crohn's disease [54].

Many studies conclude that the system should gather the following requirements to achieve suitable passive targeting [55].

1 Particle size: the optimal size to achieve adherence to intestinal mucus must be less than 10 μm, which would be interesting for the treatment of outbreak phases of IBD. To achieve incorporation into inflamed tissue and thus increase the residence time of the drug and its concentration, and given the overexpression of dendritic cells and macrophages (GALT system), the size should be less than 4 μm (ideal in maintenance treatment).
2 Polymers: chitosans, alginates, CAP, PLA and PLGA fundamentally.
3 Coating: to avoid accumulation of the system in high parts of the TGI the system must be coated with Eudragit L y S100.
4 Net charge: due to its functional groups, the gel mucus layer exhibits negative charge. In this sense, cationic systems are able to increase drug concentration over the mucin surface. This fact is critical in IBD, characterized by exacerbated motility. In any case, the charge must be less than +20 mV to prevent the system from interacting excessively and not transfer to the cell surface. It has been reported that anionic systems lower than 200 nm are able to overcame the mucosal barrier. Once on the cell surface it would interact to a greater degree with inflamed areas due to its higher protein content of eosinophils and transferrin with net positive charge.

A variety of approaches are used to deliver drugs locally into the colon via GIT, including time and pH-based systems, enzyme triggered systems and pressure based systems. However, the most effective and specific ones are those methods which rely on biologic principles, such as the methods involving lectins [54].

One of the most important strategies for improving the DDSs via GIT is drug targeting to specific sites of action. This technique not only improves drugs' pharmacokinetics helping to overcome the hurdles towards peroral drug delivery [55], but also shows several advantages for the patients. The most important ones are the prevention from side effects on healthy tissues and the increase in drug uptake by targeted cells, which permits to reduce the drug dosage [53].

The recognition of the affected tissue and the release of the drug at its site of action are allowed by biorecognitive ligands, which possess high affinity to receptors expressed in those biological locations. For that purpose, immunology offers the antibody–antigen interaction, and glycobiology provides the interaction between carbohydrates and sugar binding proteins known as lectins [55].

The lectins possess bioadhesive properties that prolong the residence time and favor the cession of the drug thanks to its union to the carbohydrates overexpressed in the cellular surface [50].

Different investigations have shown that M cells are capable of internalizing inert particles whereby the encapsulation of drugs in particles has been extended to protect them from luminal factors that degrade them and limit their absorption [56,57].

In the figure on the left, a general scheme of the intestinal organised mucosa-associated lymphoid tissue (O-MALT) (Fig. 2) is observed, which shows follicle-associated epithelium (FAE) and a Peyer's patch lymphoid follicle. The main difference between FAE and villis epithelium is the presence of M cells in FAE (figure on the right). The M cells morphology is based on the presence of microvilli in the apical membrane and a basolateral cytoplasmic invagination that forms a pocket, which may contain lymphocytes and macrophages. Some antigens and particles can follow the transcellular pathway to pass through the M cells, since these cells possess greater capacity of endocytosis and transcytosis than the absorbent enterocytes, and it is known that many microorganisms and macromolecules are able to selectively adhere to the M cells surface [58].

Lectins have been shown to enhance drug and particle adsorption in intestinal epithelial cells [15]. The binding of lectins to M cells depends on the species and the binding site, and there are also differences among M cells of the same individual [59]. To date, the only identified lectins capable of selectively binding to the surface

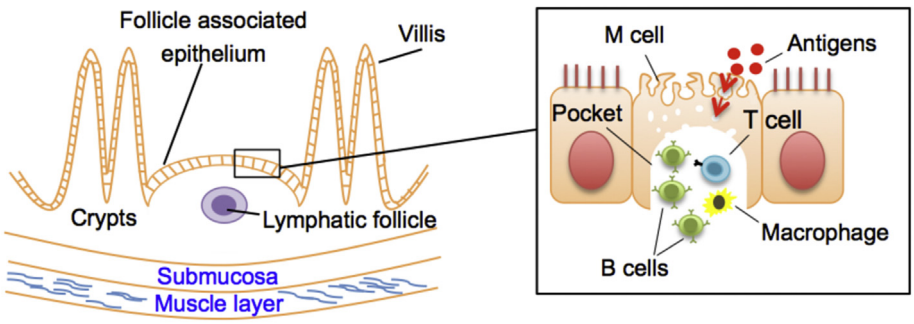

Fig. 2. General scheme of the intestinal organised mucosa-associated lymphoid tissue.

of human intestinal M cells are lectins derived from Sambucus nigra and Viscum album, which bind to the surface of human Peyer's patches (both enterocytes and M cells) [60].

Several studies have suggested the suitability of wheat germ agglutinin (WGA) from Triticum vulgare for GIT targeting. WGA, which specifically binds to sialic acid and N-acetyl-d-glucosamine, possesses cytoadhesive and cytoinvasive characteristics. Generally, this lectin is attached to drug-loaded microcarriers for enhancing the bioadhesion and prolonging the residence time in the small intestine. Upon targeting with WGA, binding to artificial human intestinal epithelium was increased considerably [61,62] Another lectins that have successfully demonstrated particle coating are the lectin from Sambucus nigra (SNA-NP) [63] and the lectin from tomato [64]. The coating of particles with lectins has become the object of numerous studies due to the remarkable growth of inflammatory bowel diseases (IBD), such as Crohn's disease or ulcerative colitis. A great limitation in the drug treatment of IBD is the delivery of the drug selectively towards the inflamed tissues. Lectin-decorated drug loaded nanoparticles (NPs) were suggested for targeting and selective adhesion to the inflamed tissue in experimental colitis. Peanut agglutinin (PNA) and WGA were covalently bound to the surface of NPs. While WGA provided a binding to the entire GIT mucosa, PNA specifically targeted to the inflamed tissue. Lectin-conjugated NPs exhibited a much higher binding and selectivity to inflamed tissue compared to plain NPs. Targeted NPs by using lectins, especially with PNA, as stable targeting moiety in the GIT appears to be a promising tool in future IBD treatment [63]. All of this makes lectin-conjugated carriers suitable for the treatment of IBD. An epithelial active targeting approach by lectins offers the potential for much more effective anti-inflammatory therapy for IBD, including drugs that are not in use in current IBD treatment because of their severe adverse effects [64] (see Table 2).

5. Other routes of administration

5.1. Lectin-mediated delivery to the gastrointestinal tract

Nasal mucosa has a small (about 150 cm^2), but highly vascularised surface area, with a relatively permeable membrane. In the nasal cavity the nasal-associated lymphoid tissue (NALT) is located. This tissue, which is covered by an epithelial layer of M-cells, is the equivalent of the GALT, showing potential for the administration of vaccines.

In order to increase the retention time in the nasal cavity, where there is a continuous mucociliary clearance, bioadhesive systems (for instance, polymers such as chitosan or carbopols) can be used. In some studies lectins are employed for this purpose. Giannasca et al. used the isolectin B4 from *Bandeiraea simplicifolia* 1 (BSI-B4) for targeting of antigens to hamster NALT and the subsequent production of specific serum IgG [65]. A few years later, Kumar et al. demonstrated that equine nasopharyngeal tonsillar tissue contains M-cells that react with a lectin from *Bandeireae simplicifolia* [66]. Another study showed that *Griffonia simplicifolia* I isolectin-B4 (GSI-B4) is almost exclusively M-cell specific for rat NALT [67].

Piazza et al. have recently studied the intranasal administration of lectin-functionalized PEG-PLGA nanoparticles for treating schizophrenia. These nanoparticles were loaded with haloperidol and decorated with *Solanum tuberosum* lectin (STL). The conjugation of the particles with STL significantly increased brain haloperidol concentrations. It might make possible the reduction of the drug dose [68].

5.2. Lectin-mediated delivery to the lungs

Lungs have a large surface area and a very thin alveolar epithelium, which makes rapid drug absorption possible. There exists evidence of lectins binding to tissues of the airway epithelium. Some of these lectins are even taken up by lung cells [69].

The alveolar epithelium consists of two cell types. The cuboidal type II cells produce the lung surfactant and act as progenitor cells for the type I cells, which cover the majority of the alveolae surface and allow the gas exchange [70]. Each cell type specifically binds to a kind of lectin: *Maclura pomifera* agglutinin binds to type II cells, while *Ricinus communis* agglutinin and TL bind to type I cells [71].

Some years ago, research about WGA-functionalised liposomes to human alveolar cells in primary culture was done, and the improved uptake obtained made these liposomes potential candidates as drug carriers for local and systemic administration [72].

Wheat germ agglutinin (WGA) has been used for the decoration of PLGA nanoparticles in order to enhance their interaction with lectin receptors on the alveolar epithelium. With this technique, researchers achieved elevated antibiotic levels in the lungs for treating tuberculosis [73,74]. It has been shown that WGA-functionalized particles are not only bound to, but also internalized by primary human alveolar epithelial cells [62].

Table 2
Main RILs used as excipients for drug targeting.

Lectin	Bioactive molecule	Target	Particle platform	Application
Ulex Europaeus I (UEA I)	–	Mouse Peyer's patch M cells	Polymerized liposome	Vaccine
	HIV peptides	Mouse Peyer's patch M cells	PLGA microparticles	Vaccine
	Hepatitis B surface antigen (HBsAg)	Mouse Peyer's patch M cells	Lectin-liposome conjugate	Vaccine
			PLGA nanoparticles	Vaccine
	Toll-like receptor (TLR)	Mouse Peyer's patch M cells	PLGA nanoparticles	Vaccine
Wheat Germ Agglutinin (WGA)		Mouse Peyer's patch M cells	Polymerized liposome	Vaccine
	β-galactosidase	Mucin coatings and Caco-2 cell monolayers.	PLGA microparticles	Lactose intolerance
	Insulin	Serum	Solid Lipid nanoparticles	Hyperglycemia
	BSA	Caco-2 cell monolayers	PLGA nanospheres	Targeting peptides, proteins, and DNA
Sambucus nigra lectin	Rhodamine isothiocyanate and 1,3-diaminopropane	Mucous membrane	PVM/MA nanoparticles	IBD
Lycopersicon esculentum (tomato) (TL)	BSA	The everted gut sac model	Polystyrene microspheres	IBD
	Fluorescent	Mouse Peyer's patch M cells	Polystyrene nanoparticles	Quantification of the bioadhesive phenomenon
Galactose ligands	Paclitaxel	HepG2 tumor cells	Galactosamine-conjugated micelles	Cancer therapy
Aleuria aurantia lectin	NCBBH	Two colorectal cancer cell lines	–	Cancer therapy

Table 3
Lectins described and their use in drug targeting.

Lectin	Specificity	Type of targeting
Arachis hypogaea lectin	GalNAc	Oral cavity
Canavalia ensiformis lectin		Oral cavity
Maackia amurensis agglutinin	NeuNAcα(2–3) Galβ (1–4) GlcNAc/Glc	NSCLC cells
Solanum tuberosum lectin	GlcNAC	Nasal epithelial membrane Corneal epithelia
Lycopersicum esculentum agglutinin	(GlcNAc)3	Lungs (alveolae, type I cells)
Triticum vulgaris agglutinin	(D-GlcNAc)2, NeuNAc	Lungs Oral cavity
Bandeireae simplifolia and *Griffonia simplicifolia* I isolectin B4	α-D-Gal	Nasal mucosa (M-cells of NALT)
Maclura pomifera agglutinin	GalNAc	Lungs (alveolae, type II cells)
Ricinus communis agglutinin	D-Gal	Lungs (alveolae, type I cells)

Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; NeuNAc, sialic acid; Man, mannose; Fuc, fucose.

Maackia amurensis agglutinin (MAA) is a NeuNAcα (2–3) Galβ (1–4) GlcNAc/Glc specific lectin which interacts with the cells of non-small cell lung cancer (NSCLC). Because of that, this lectin can be used in targeting NSCLC cells, reducing the side effects of drugs on healthy cells and tissues. MAA also shows the capacity of enhancing the cytotoxic effect of Paclitaxel, a chemotherapeutic drug [75].

5.3. Lectin-mediated ocular drug delivery

The most important surface tissues in the eye are the conjunctiva and the cornea. Blinking, mucin secretion and tear turnover make ocular drug absoption difficult. The contact between the drug and the corneal and conjunctival epithelia can be prolonged by ocular bioadhesive lectins, consequently enhancing drug absorption. Schaeffer et al. observed that after treating rabbit corneas with WGA, the binding of ganglioside-containing liposomes was enhanced [76]. Another recent study revealed the utility of *Solanum tuberosum* lectin in targeting rat and rabbit precorneal tissues in *ex-vivo* experiments since the lectin shows specificity of binding to N-acetyl-D-glucosamine. Therefore, proper lectins may be used in the development of non-irritant and safe ocular drug delivery systems [77].

5.4. Lectin-mediated drug targeting to the oral cavity

A range of diseases that require local therapy occur within the oral cavity: dental caries, gingivitis, periodontal disease, candidosis, xerostoma ('dry mouth'), aphthous ulceration and oral carcinomas. Lectins can be used to increase the retention of drugs in the oral cavity since they show affinity for oral mucosal cells [78]. Smart et al. demonstrated that the lectins from *Arachis hypogaea*, *Canavalia ensiformis* and *Triticum vulgaris* were retained on oral mucosal tissue. The latter one showed the greatest levels of binding [79].

The binding of lectins from *T. vulgaris* and *A. hypogaea* to epithelial surfaces was established by an in vivo study in humans. Both lectins appeared to persist in the oral cavity for several hours [80]. In another study, *C. ensiformis* lectin-decorated liposomes containing metronidozole showed potential to be retained in periodontal pockets and deliver the drug during prolonged periods [81] (see Table 3).

6. Conclusion

Lectins have increasingly gained interest in pharmaceutical technology as active excipients to modulate release of associated drug. In this sense, several research groups are focus on construction of immunotoxins or conjugates. Specific lectins and also B-chain of RILs, has been used for that purpose. RILs A-chain is also interesting as toxic moiety of these systems or to develop new inflammatory animal model. Reverse lectin-targeting has been proposed as an useful strategy for oral or other routes, controlled released, as well as diagnostic tools with important clinical applications. Prospective research in this field, looking for new targets for the known lectins and finding for new lectins, should be an interesting challenge for pharmaceutical technology researchers.

References

[1] A. Varki, M.E. Etzler, R.D. Cummings, J.D. Esko, Discovery and classification of glycan-binding proteins (chapter 26), in: A. Varki, M.E. Etzler, R.D. Cummings, J.D. Esko (Eds.), Essentials of Glycobiology, Cold Spring Harbor Laboratory Press, New York, 2009.

[2] Reconocimiento de hidratos de carbono: las lectinas. http://themedicalbiochemistrypage.org/es/glycoproteins-sp.php.

[3] V. Gajbhiye, S. Gong, Lectin functionalized nanocarriers for gene delivery, Biotechnol. Adv. 31 (2013) 552–562.

[4] C. Bies, C.M. Lehr, J.F. Woodley, Lectin-mediated drug targeting: history and applications, Adv. Drug Deliv. Rev. 56 (2004) 425–435.

[5] N. Sharon, H. Lis, History of lectins: from hemagglutinins to biological recognition molecules, Glycobiology 14 (11) (2004) 53–62.

[6] N. Sharon, Lectins: past, present and future, Biochem. Soc. Trans. 36 (6) (2008) 1457–1460.

[7] R. Bawa, G.F. Audette, I. Rubinstein, Reverse lectin targeting, in: R. Bawa, G.F. Audette, I. Rubinstein (Eds.), Handbook of Clinical Nanomedicine: Nanoparticles, Imaging, Therapy, and Clinical Applications, Taylor and Francis Group, New York (, 2016, pp. 1548–1561.

[8] F. Gabor, E. Bogner, A. Weissenboeck, M. Wirth, The lectin–cell interaction and its implications to intestinal lectin-mediated drug delivery, Adv. Drug Deliv. Rev. 56 (2004) 459–480.

[9] H. Gupta, D. Bhandari, A. Sharma, Recent trends in oral drug delivery: a review, Recent Pat. Drug Deliv. Formul. 3 (2009) 162–173.

[10] D.R. Friend, Targeted gastrointestinal delivery, in: E. Touitou, B.W. Barry (Eds.), Enhancement in Drug Delivery, Taylor and Francis Group, New York, 2006, pp. 69–80.

[11] J. Perez-Vilar, Gastrointestinal mucus gel barrier, in: A. Bernkop-Schnürch (Ed.), Oral Delivery of Macromolecular Drugs: Barriers, Strategies and Future Trends, Springer, New York, 2009, pp. 21–30.

[12] L.M. Ensign, R. Cone, J. Hanes, Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers, Adv. Drug Deliv. Rev. 64 (2012) 557–570.

[13] N.L. Perillo, M.E. Marcus, L.G. Baum, Galectins: versatile modulators of cell adhesion, cell proliferation, and cell death, J. Mol. Med. 76 (1998) 402–412.

[14] V. Sihorkar, S.P. Vyas, Potential of polysaccharide anchored liposomes in drug delivery, targeting and immunization, J. Pharm. Pharm. Sci. 4 (2001) 138–158.

[15] N. Nagy, Y. Bronckart, I. Camby, H. Legendre, H. Lahm, H. Kaltner, Y. Hadari, P.V. Ham, P. Yeaton, J.C. Pector, Y. Zick, I. Salmon, A. Danguy, R. Kiss, H.J. Gabius, Galectin-8 expression decreases in cancer compared with normal and dysplastic human colon tissue and acts significantly on human colon cancer cell migration as a suppressor, Gut 50 (2002) 392–401.

[16] T. Minko, Drug targeting to the colon with lectins and neoglycoconjugates, Adv. Drug Deliv. Rev. 56 (2004) 491–509.

[17] H. Li, W. Donga, J. Zhoua, X. Xub, F. Li, Triggering effect of N-acetylglucosamine on retarded drug release from a lectin anchored chitosan nanoparticles-in-microparticles system, Int. J. Pharm. 449 (2013) 37–43.

[18] M.G. Batelli, S. Musiani, L. Buonamici, S. Santi, M. Rccio, N.M. Maraldi, T. Girbes, F. Stirpe, Interaction of volkensin with HeLa cells: binding, uptake, intracellular localization, degradation and exocytosis, Cell. Mol. Life Sci. 61 (2004) 1975–1984.

[19] T. Girbés, J.M. Ferreras, F.J. Arias, F. Stirpe, Description, distribution, activity and phylogenetic relationship of ribosome-inactivating proteins in plants, fungi and bacteria, Mini Rev. Med. Chem. 4 (2004) 461–476.

[20] M. Garrosa, P. Jiménez, J. Tejero, P. Cabrero, D. Cordoba-Diaz, E.J. Quinto, M.J. Gayoso, T. Girbés, Toxicity of the anti-ribosomal Lectin Ebulin f in lungs and intestines in elderly mice, Toxins (Basel) 7 (2015) 367–379.

[21] F. Stirpe, Ribosome-inactivating proteins: from toxins to useful proteins, Toxicon 67 (2013) 12–16.
[22] F. Stirpe, M.G. Battelli, Ribosome-inactivating proteins: progress and problems, Cell. Mol. Life Sci. 63 (2006) 1850–1866.
[23] B. Barriuso, P. Antolín, F.J. Arias, A. Girotti, P. Jiménez, M. Cordoba-Diaz, D. Cordoba-Diaz, T. Girbés, Anti-human endoglin (hCD105) immunotoxin-containing recombinant single chain ribosome-inactivating protein musarmin 1, Toxins (Basel) 8 (2016) E184.
[24] U. Cavallaro, A. del Vecchio, D.A. Lappi, M.R. Soria, A conjugate between human urokinase and saporin, a type-1 ribosome-inactivating protein, is selectively cytotoxic to urokinase receptor-expressing cells, J. Biol. Chem. 268 (1993) 23186–23190.
[25] J. Benitez, J.M. Ferreras, R. Muñoz, Y. Arias, R. Iglesias, M. Cordoba-Diaz, R. del Villar, T. Girbes, Cytotoxicity of an ebulin-l-anti-human CD105 immunotoxin on mouse f ibroblasts (L929) and rat myoblast (L6E9) cells expressing human CD105, Med. Chem. 1 (2005) 65–70.
[26] J.M. Ferreras, L. Citores, R. Iglesias, P. Jiménez, T. Girbés, Use of ribosome-inactivating proteins from *Sambucus* for the construction of immunotoxins and conjugates for cancer therapy, Toxins (Basel) 3 (2011) 420–441.
[27] T. Girbés, L. Citores, J.M. Ferreras, M.A. Rojo, R. Iglesias, R. Muñoz, F.J. Arias, M. Calonge, J.R. García, E. Méndez, Isolation and partial characterization of nigrin b, a non-toxic novel type 2 ribosome-inactivating protein from the bark of *Sambucus nigra* L, Plant Mol. Biol. 22 (1993) 1181–1186.
[28] T. Girbés, L. Citores, R. Iglesias, J.M. Ferreras, R. Muñoz, M.A. Rojo, F.J. Arias, J.R. García, E. Méndez, M. Calonge, Ebulin l, a nontoxic novel type 2 ribosome-inactivating protein from *Sambucus ebulus* L. leaves, J. Biol. Chem. 268 (1993) 18195–18199.
[29] P. Jimenez, P. Cabrero, J.E. Basterrechea, J. Tejero, D. Cordoba-Diaz, T. Girbes, Isolation and molecular characterization of two lectins from dwarf elder (*Sambucus ebulus* L.) blossoms related to the Sam n1 allergen, Toxins (Basel) 5 (2013) 1767–1779.
[30] R. Muñoz, Y. Arias, J.M. Ferreras, M.A. Rojo, M.J. Gayoso, M. Nocito, J. Benitez, P. Jiménez, C. Bernabéu, T. Girbés, Targeting a marker of the tumour neovasculature using a novel anti-human CD105-immunotoxin containing the non-toxic type 2 ribosome-inactivating protein nigrin b, Cancer Lett. 256 (2007) 73–80.
[31] R. Muñoz, Y. Arias, J.M. Ferreras, P. Jiménez, M.A. Rojo, C. Bernabéu, D. Córdoba-Díaz, T. Girbés, Transient injury-dependent up-regulation of CD105 and its specific targeting with an anti-vascular anti-mouse endoglin-nigrin b immunotoxin, Med. Chem. 8 (2012) 996–1002.
[32] R. Muñoz, Y. Arias, J.M. Ferreras, P. Jiménez, C. Langa, M.A. Rojo, M.J. Gayoso, D. Córdoba-Díaz, C. Bernabéu, T. Girbés T, In vitro and in vivo effects of an anti-mouse endoglin (CD105)-immunotoxin on the early stages of mouse B16MEL4A5 melanoma tumours, Cancer Immunol. Immunother. 62 (2013) 541–551.
[33] J.M. Pascal, P.J. Day, A.F. Monzingo, S.R. Ernst, J.D. Robertus, R. Iglesias, Y. Pérez, J.M. Férreras, L. Citores, T. Girbés, 2.8-A crystal structure of a nontoxic type-II ribosome-inactivating protein, ebulin l, Proteins 43 (2001) 319–326.
[34] M. Bortolotti, A. Bolognesi, M.G. Battelli, L. Polito, High in vitro anti-tumor efficacy of dimeric rituximab/saporin-S6 immunotoxin, Toxins (Basel) 21 (2016) E192.
[35] S. Santanché, A. Bellelli, M. Brunori, The unusual stability of saporin, a candidate for the synthesis of immunotoxins, Biochem. Biophy. Res. Commun. 234 (1997) 129–132.
[36] J. Tejero, P. Jiménez, E.J. Quinto, D. Cordoba-Diaz, M. Garrosa, M. Cordoba-Diaz, M.J. Gayoso, T. Girbés, Elderberries: a source of ribosome-inactivating proteins with lectin activity, Molecules 20 (2015) 2364–2387.
[37] P. Jiménez, J. Tejero, D. Cordoba-Diaz, E.J. Quinto, M. Garrosa, M.J. Gayoso, T. Girbés, Ebulin from dwarf elder (*Sambucus ebulus* L.): a mini-review, Toxins (Basel) 7 (2015) 648–658.
[38] R. Gilabert-Oriol, A. Weng, B. Mallinckrodt, M.F. Melzig, H. Fuchs, M. Thakur, Immunotoxins constructed with ribosome-inactivating proteins and their enhancers: a lethal cocktail with tumor specific efficacy, Curr. Pharm. Des. 20 (2014) 6584–6643.
[39] L. Citores, J.M. Ferreras, R. Muñoz, J. Benítez, P. Jiménez, T. Girbés, Targeting cancer cells with transferrin conjugates containing the non-toxic type 2 ribosome-inactivating proteins nigrin b or ebulin l, Cancer Lett. 184 (2002) 29–35.
[40] M.S. Mohamed, S. Veeranarayanan, A.C. Poulose, Y. Nagaoka, H. Minegishi, Y. Yoshida, T. Maekawa, D.S. Kumar, Type 1 ribotoxin-curcin conjugated biogenic gold nanoparticles for a multimodal therapeutic approach towards brain cancer, Biochim. Biophys. Acta 1840 (2014) 1657–1669.
[41] F. Cominelli, K.O. Arseneau, A. Rodriguez-Palacios, T.T. Pizarro, Uncovering pathogenic mechanisms of inflammatory bowel disease using mouse models of Crohn's disease-like ileitis: what is the right model? Cell. Mol. Gastroenterol. Hepatol. 4 (2017) 19–32.
[42] M.J. Lord, N.A. Jolliffe, C.J. Marsden, C.S. Pateman, D.C. Smith, R.A. Spooner, P.D. Watson, L.M. Ro-berts, Ricin. Mechanisms of cytotoxicity, Toxicol. Rev. 22 (2003) 53–64.
[43] M.J. Gayoso, R. Muñoz, Y. Arias, R. Villar, M.A. Rojo, P. Jimenez, J.M. Ferreras, I. Aranguez, T. Girbes, Specific dose-dependent damage of Lieberkühn crypts promoted by large doses of type 2 ribosome-inactivating protein nigrin b intravenous injection to mice, Toxicol. Appli. Pharmacol. 207 (2005) 138–146.
[44] T. Sato T, J.H. van Es, H.J. Snippert, D.E. Stange, R.G. Vries, M. van den Born, N. Barker, N.F. Shroyer, M. van den Wetering, H. Clevers, Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts, Nature 469 (2011) 415–418.
[45] T.M. Yeung, L.A. Chia, C.M. Kosinski, C.J. Kuo, Regulation of self-renewal and differentiation by the intestinal stem cell niche, Cell. Mol. Life Sci. 68 (2011) 2513–2523.
[46] P. Jimenez, M.J. Gayoso, M. Garrosa, D. Cordoba-Diaz, P. Cabrero, J. Tejero, M. Aracil, T. Girbes, Paneth cells are also target of the ribotoxic lectin nigrin b, Histol. Histopathol. 29 (2014) 1057–1063.
[47] P. Jimenez, D. Cordoba-Diaz, P. Cabrero, M. Aracil, M.J. Gayoso, M. Garrosa, M. Cordoba-Diaz, T. Girbes, Plasma accumulations of vitamin B6 from an oral dose in a new reversible model for mouse gut injury and regeneration, Food Nutr. Sci. 4 (2013) 908–917.
[48] D.R. Friend, Targeted gastrointestinal delivery, in: E. Touitou, B.W. Barry (Eds.), Enhancement in Drug Delivery, Taylor and Francis Group, New York, 2006, pp. 69–80.
[49] U. Agrawal, R. Sharma, N. Mody, S. Dubey, S.P. Vyas, Improved oral bioavailability of bioactives through lipid-based nanoarchitectures, in: A.M. Grumezescu (Ed.), Surface Chemistry of Nanobiomaterials: Applications of nanobiomaterials, Elsevier, 2016, pp. 433–437.
[50] H. Gupta, D. Bhandari, A. Sharma, Recent trends in oral drug delivery: a review, Recent Pat. Drug Deliv. Formul. 3 (2009) 162–173.
[51] D.R. Friend, Targeted gastrointestinal delivery, in: E. Touitou, B.W. Barry (Eds.), Enhancement in Drug Delivery, Taylor and Francis Group, New York, 2006, pp. 69–80.
[52] J. Perez-Vilar, Gastrointestinal mucus gel barrier, in: A. Bernkop-Schnürch (Ed.), Oral Delivery of Macromolecular Drugs: Barriers, Strategies and Future Trends, Springer, New York, 2009, pp. 21–30.
[53] L.M. Ensign, R. Cone, J. Hanes, Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers, Adv. Drug Deliv. Rev. 64 (2012) 557–570.
[54] T. Minko, Drug targeting to the colon with lectins and neoglycoconjugates, Adv. Drug Deliv. Rev. 56 (2004) 491–509.
[55] F. Gabor, E. Bogner, A. Weissenboeck, M. Wirth, The lectin–cell interaction and its implications to intestinal lectin-mediated drug delivery, Adv. Drug Deliv. Rev. 56 (2004) 459–480.
[56] G. Ratzinger, X. Wang, M. Wirth, Targeted PLGA microparticles as a novel concept for treatment of lactose intolerance, J. Control. Release 147 (2010) 187–192.
[57] F. Roth-Walter, I. Schöll, E. Untersmayr, Mucosal targeting of allergen-loaded microspheres by Aleuria aurantia lectin, Vaccine 23 (2005) 2703–2710.
[58] N. Hussein, P.U. Jani, A. Florence, Enhanced oral uptake of tomato lectin-conjugated nanoparticles in the rat, Pharm. Res. 14 (5) (1997) 613–618.
[59] B. Naisbett, J. Woodley, Binding of tomato lectin to the intestinal mucosa and its potential for oral drug delivery, Biochem. Soc. Trans. 18 (5) (1990) 869–880.
[60] B. Moulari, A. Béduneau, Y. Pellequer, A. Lamprecht, Lectin decorated nanoparticles enhance binding to the inflamed tissue in experimental colitis, J. Control. Release 188 (2014) 9–17.
[61] C. Lautenschläger, C. Schmidt, D. Fischer, A. Stallmach, Drug delivery strategies in the therapy of inflammatory bowel disease, Adv. Drug Deliv. Rev. 71 (2014) 58–76.
[62] C.M. Lehr, Lectin-mediated drug delivery: the second generation of bioadhesives, J. Control. Release 65 (2000) 19–29.
[63] S.C. Diesner, X. Wang, E. Jensen-Jarolim, E. Untersmayr, F. Gabor, Use of lectin-functionalized particles for oral immunotherapy, Ther. Deliv. 3 (2) (2012) 277–290.
[64] M.A. Clark, B.H. Hirst, M.A. Jepson, Lectin-mediated mucosal delivery of drugs and microparticles, Adv. Drug Deliv. Rev. 43 (2000) 207–223.
[65] P.J. Giannasca, J.A. Boden, T.P. Monath, Targeted delivery of antigen to hamster nasal lymphoid tissue with M-cell-directed lectins, Infect. Immun. 65 (1997) 4288–4298.
[66] P. Kumar, J.F. Timoney, A.S. Sheoran, M cells and associated lymphoid tissue of the equine nasopharyngeal tonsil, Equine Vet. J. 33 (2001) 224–230.
[67] S. Takata, O. Ohtani, Y. Watanabe, Lectin binding patterns in rat nasal-associated lymphoid tissue (NALT) and the influence of various types of lectin on particle uptake in NALT, Arch. Histol. Cytol. 63 (2000) 305–312.
[68] J. Piazza, T. Hoare, L. Molinaro, K. Terpstra, J. Bhandari, P.R. Selvaganapathy, B. Gupta, R.K. Mishra, Haloperidol-loaded intranasally administered lectin functionalized poly (ethylene glycol)–block-poly(D,L)-lactic-co-glycolic acid (PEG–PLGA) nanoparticles for the treatment of schizophrenia, Eur. J. Pharm. Biopharm. 87 (2014) 30–39.
[69] S.M. Yi, R.E. Harson, J. Zabner, M.J. Welsh, Lectin binding and endocytosis at the apical surface of human airway epithelia, Gene Ther. 8 (2001) 1826–1832.
[70] C. Barkauskas, M. Cronce, C. Rackley, E. Bowie, D. Keene, B. Stripp, S. Randell, P. Noble, B. Hogan, Type 2 alveolar cells are stem cells in adult lung, J. Clin. Invest. 123 (2013) 3025–3036.
[71] P.W. Bankston, G.A. Porter, A.J. Milici, G.E. Palade, Differential and specific labeling of epithelial and vascular endothelial cells of the rat lung by *Lycopersicon esculentum* and *Griffonia simplicifolia* lectins, Eur. J. Cell Biol. 54 (1991) 187–195.
[72] A. Bruck, R. Abu-Dahab, G. Borchard, U.F. Schafer, C.M. Lehr, Lectin-functionalized liposomes for pulmonary drug delivery: interaction with human alveolar epithelial cells, J. Drug Target 9 (2001) 241–251.
[73] J.C. Sung, B.L. Pulliam, D.A. Edwards, Nanoparticles for drug delivery to the lungs, Trends Biotechnol. 25 (2007) 563–570.

[74] A. Sharma, S. Sharma, G.K. Khuller, Lectin-functionalized poly (lactide-co-glycolide) nanoparticles as oral/aerosolized antitubercular drug carriers for treatment of tuberculosis, J. Antimicrob. Chemother. 54 (2004) 761–766.
[75] R.C. Lalli, K. Kaur, S. Dadsena, A. Chakraborti, R. Srinivasan, S. Ghosh, Maackia amurensis agglutinin enhances paclitaxel induced cytotoxicity in cultured non-small cell lung cancer cells, Biochimie 115 (2015) 93–107.
[76] H.E. Schaeffer, J.M. Breitfeller, D.L. Krohn, Lectin-mediated attachment of liposomes to cornea: influence on transcorneal drug flux, Invest. Ophthalmol. Vis. Sci. 23 (1982) 530–533.
[77] M. Gavrovic-Jankulovic, R. Prodanovic, Drug delivery: plant lectins as bioadhesive drug delivery systems, J. Biomat. Nanobiotech. 2 (2011) 614–621.
[78] J.D. Smart, Lectin-mediated drug delivery in the oral cavity, Adv. Drug Deliv. Rev. 56 (2004) 481–489.
[79] J.D. Smart, P.K.K. Nantwi, K.L. Green, D.J. Rogers, A quantitative evaluation of radiolabelled lectin retention on oral mucosa in vitro and in vivo, Eur. J. Pharm. Biopharm. 53 (2002) 289–292.
[80] R.J. Gibbons, I. Dankers, Association of food lectins with human oral epithelial cells in vivo, Arch. Oral Biol. 28 (1983) 561–566.
[81] S.P. Vyas, V. Sihorkar, P.K. Dubey, Preparation, characterization and in vitro antimicrobial activity of metronidazole-bearing lectinized liposomes for intraperidontal pocket delivery, Pharmazie 56 (2001) 554–560.