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Abstract

Nasal administration offers a possibility of delivering drugs to the brain. In the present work, nasal drug delivery systems were designed based on cationic Eudragit® EPO (EPO) and anionic Eudragit® L100-55 (L100-55) methacrylate copolymers. Two types of nanocarriers were prepared using interpolyelectrolyte complexation between these polymers. The first type of nanoparticles was prepared by forming interpolyelectrolyte complexes between unmodified EPO and L100-55. The second type of nanoparticles was formed through the complexation between PEGylated L100-55 and EPO. For this purpose, PEGylated L100-55 was synthesized by chemical conjugation of L100-55 with O-(2-aminoethyl)polyethylene glycol. The mucoadhesive properties of these nanoparticles were evaluated *ex vivo* using sheep nasal mucosa. Nanoparticles based on EPO and L100-55 exhibited mucoadhesive properties towards nasal mucosa, whereas PEGylated nanoparticles were non-mucoadhesive hence displayed mucus-penetrating properties. Both types of nanoparticles were used to formulate haloperidol and their ability to deliver the drug to the brain was evaluated in rats *in vivo*.

Keywords: nasal drug delivery, nose-to-brain delivery, Eudragit®, interpolyelectrolyte complex, nanoparticles, mucoadhesion

The treatment of brain diseases (e.g. neurodegenerative and psychiatric conditions, epilepsy, oncology, etc.) is a very challenging task due to the presence of the blood-brain and the blood-cerebrospinal fluid barriers, which prevent drug penetration into the brain.[1-3] Nasal drug administration is considered as a promising approach to bypass these barriers.[4,5] This route minimizes systemic side effects and shortens the onset of drug action.[6] However, the presence of mucociliary clearance, which leads to rapid removal of drug molecules from the nasal cavity, may hamper their efficient penetration to the brain. Mucosal blanket in the nasal cavity also acts as a barrier preventing diffusion of drugs into epithelial cells.

Commonly used approaches to improve the efficiency of drugs administered via nasal route include mucoadhesive dosage forms[7] and carriers with enhanced mucus-penetrating properties.[8] Mucoadhesive dosage forms are typically designed using cationic and anionic polymers capable of interacting with mucosal surfaces.[9] For example, mucoadhesive properties of cationic chitosan and its derivatives are widely used in the design of dosage forms for nasal drug delivery.[10,11] Eudragit® EPO is a synthetic cationic terpolymer of N,N-dimethylaminoethyl methacrylate, methylmethacrylate and butylmethacrylate (Figure 1S), exhibiting mucoadhesive properties. EPO and its acrylated derivatives have previously been considered as mucoadhesive materials for nasal drug delivery.[12] Eudragit® L100-55 is an anionic copolymer of methacrylic acid with ethylacrylate (Figure 1S) that was also considered as a mucoadhesive excipient, including the preparation of its thiolated derivatives.[13] A combination of oppositely-charged Eudragits® in solutions results in formation of interpolyelectrolyte complexes (IPEC), which can potentially be used in the design of various formulations for drug delivery.[14,15]

PEGylation or decoration of dosage form surfaces with oligomeric poly(ethylene glycol) (PEG) is increasingly being used as an approach to optimise the performance of many drug delivery systems.[16,17] PEGylated nanoparticles demonstrated increased systemic

circulation time, improved pharmacokinetics and pharmacodynamics parameters and limiting immunogenic and antigenic reactions.[18,19] Nanoparticles decorated with PEG are also widely used in transmucosal drug delivery as they exhibit mucus-penetrating properties. There are reports on application of PEGylated nanoparticles in nasal,[8,20] ocular,[21,22] gastrointestinal,[23,24] and vaginal[25,26] drug delivery.

In the majority of studies on the design of PEGylated nanocarriers these were prepared either through the use of block-copolymers containing PEG as one of the blocks,[27] or through stabilisation of nanoparticles using PEG-containing emulsifying or surface-active agents such as Pluronics®,[28] or through direct conjugation of PEG to functionalised surfaces,[29] or through the use of functionalised phospholipids resulting in PEGylated liposomes.[30] Here we propose a new alternative approach, which is based on self-assembly of oppositely charged polymers to form IPECs, where one of the polymers is PEGylated.

In this work, PEGylated derivatives of Eudragit® L100-55 (L100-55) were synthesised by reaction of its carboxylic groups with O-(2-aminoethyl)polyethylene glycol. PEGylated and non-PEGylated nanoparticles were prepared based on IPECs of Eudragit® EPO and L100-55. To the best of our knowledge, this is the first study reporting the design of PEGylated nanoparticles based on polyelectrolyte complexes formed by oppositely-charged Eudragits®. Both PEGylated and non-PEGylated nanoparticles developed in this study were evaluated as nanocarriers to deliver a model psychoactive drug haloperidol to the brain both *ex vivo* and *in vivo*. A minimally invasive *in vivo* model was established for the studies of haloperidol.

Materials and Methods

Description of materials and some preparative and instrumental methods can be found in Supplementary information.

In vitro retention studies

The retention of the nanoparticles on nasal mucosal tissue was studied according to the protocol previously developed and described by the Khutoryanskiy group.[12,31-34] The detailed description is given in Supplementary information.

In vivo studies

These experiments were approved by the Ethical committee of Kazan State Medical University (approval № 8 from 30th October 2018). Animals were kept in standard animal house conditions with a natural light-dark cycle and fed with a multi-ration pellets for rodents. Animals had unlimited access to water. In this study, 18 healthy 3 to 4 month-old male Wistar rats (mean weight 300-400 g) were used. Each sample was tested in 6 animals, for control a commercial formulation of haloperidol (5 mg/mL haloperidol sterile solutions containing lactic acid, Ozone Pharmaceutical Ltd, Russian Federation) diluted to concentration 1 mg/mL with deionised water was used. Experiments in rats were conducted according to the protocol

described by Natfji et al.[35] with minor changes. Nanoparticles dispersion or control (concentration of haloperidol in all samples was 1 mg/kg of rat weight) were administered into rat nostrils using a specially designed plastic cannula within 5 minutes, then each rat was placed in a special installation for assessing catalepsy (OpenScience, Russia) with a horizontal plastic bar located at a height of 10 cm. The animals were gently placed on a bar at 5, 10, 20, 30, 60, 120 and 180 min after nasal administration of haloperidol formulations. If the rat was still standing on a bar for more than 3 minutes, it was gently removed from it. The time spent by each rat on the bar was recorded. After each experiment, the animals were immediately returned to their cages in the animal house.

Results

Synthesis and Characterization of PEGylated L100-55

It has been established by many researchers that nanoparticles, whose surfaces are decorated with short-chain PEG exhibit enhanced penetration through the mucus layer.[21,36-38] To demonstrate this possibility for interpolyelectrolyte complexes based on Eudragit® copolymers, PEGylated L100-55 was synthesised. PEGylation of L100-55 was carried out in an aqueous medium, with *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride and N-hydroxysuccinimide used as catalysts. The reaction scheme shown in Figure 1.

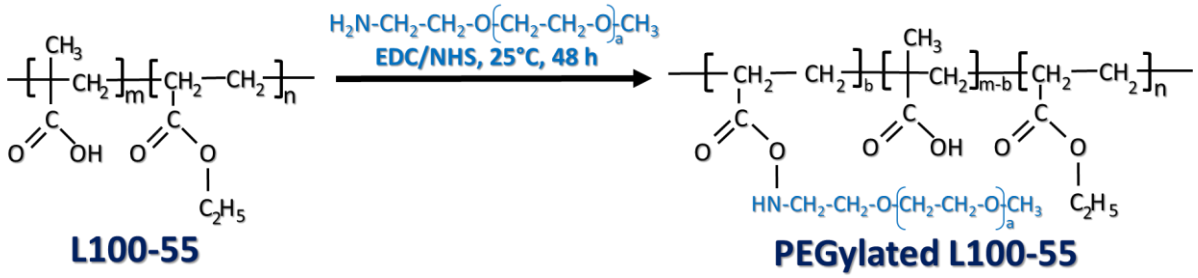
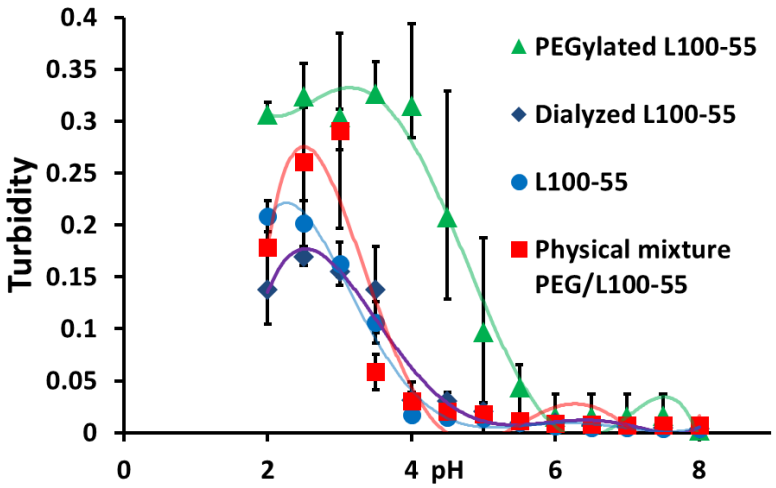


Figure 1. Synthesis of PEGylated L100-55.

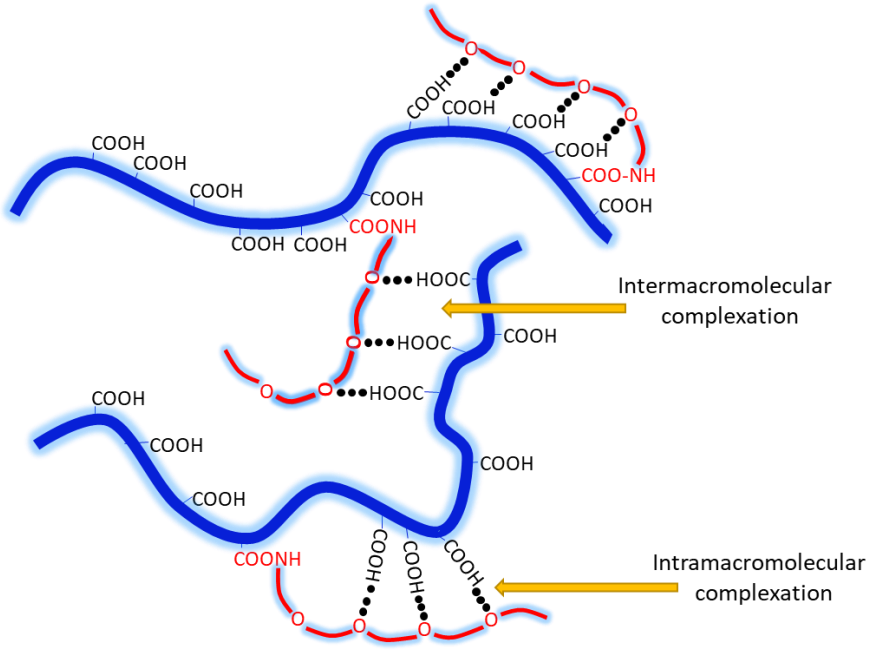
We have also prepared the dialyzed form of L100-55 for comparative analysis with original L100-55 and PEGylated L100-55. The successful modification of L100-55 through PEGylation was confirmed with various physicochemical methods, including FTIR spectroscopy, differential scanning calorimetry and elemental analysis. These results are presented and discussed in Supplementary information.

The effects of solution pH on the solubility of PEGylated polymer as well as the dialyzed L100-55 and parent L100-55 were studied using turbidity measurements (Figure 2a). L100-55 is fully soluble and forms transparent solution in a board range of pHs from 8.0 to 5.5, however at pH < 5.5 it begins to form turbid solution, aggregate and eventually precipitate, which is in agreement with previous reports.[39] Similar behavior is observed in the case of dialyzed L100-55. However, the pH of transition from soluble to insoluble state for PEGylated L100-55 is shifted to the right. PEGylated L100-55 starts to aggregate at pH < 6.0 and the maximal turbidity value is observed at pH < 3.0. We also studied the pH dependence of turbidity for the physical mixture of L100-55 with PEG, which did not demonstrate any significant difference from L100-55 and dialyzed L100-55. Previously, Peppas and co-workers[40,41] have reported the possibility of intramacromolecular complexation in hydrogels composed of graft copolymers of poly(methacrylic acid) and PEG via hydrogen bonding. We hypothesize that similar possibility for intra- and inter-macromolecular hydrogen-bonded complexes is also possible in our PEGylated L100-55. The shift of turbidimetric titration curve of PEGylated L100-55 is likely due to the formation of intra- and inter-macromolecular complexes between grafted chains of PEG and carboxylic groups of L100-55 according to the scheme shown in Figure 2b. The lack of this effect in physical mixtures of L100-55 and PEG could be explained by relatively low molecular weight of PEG used (10 kDa); however, when

PEG chains of this molecular weight are directly grafted to L100-55 the complexation via hydrogen bonding becomes more pronounced.



(a)



(b)

Figure 2. (a) Turbidity of L100-55, dialyzed L100-55 and PEGylated L100-55 solutions at different pHs (*n*=3; mean values ±SD); (b) Proposed schematic of intermacromolecular and intramacromolecular complexation.

Preparation and Characterization of IPEC Nanoparticles

The possibility of interaction between terpolymer EPO and L100-55 has previously been reported by Moustafine et al. [39] In this study, we have used the ability of these polyelectrolytes to form IPEC with the aim of preparing nanoparticles with and without PEGylated surface.

Initially, we have determined the optimal ratio of copolymers that could be used to form colloidally-stable nanoparticles using dynamic light scattering. The characteristics of IPECs prepared from EPO / L100-55 and EPO / PEGylated L100-55 in different polymer ratios are summarised in Table 1 immediately after polymer mixing and following 7 days of sample storage.

Table 1. Characteristics of IPECs.

Sample	Ratio of polymers	Immediately			After 7 days	
		Diameter, nm	PDI	Zeta-potential, mV	Diameter, nm	PDI
EPO / L100 - 55	1:1	143 ± 1	0.21	25.1±0.9	141 ± 2	0.271
	1:0.5	130 ± 2	0.226	33.3±1.0	116 ± 2	0.217
	0.5:1	123 ± 2	0.182	0.0±3.3	122 ± 2	0.229
EPO /	1:1	123 ± 3	0.228	17.3±0.8	160 ± 1	0.237

PEGylated	1:0.5	110 ± 1	0.227	16.6±1.0	110 ± 1	0.227
L100 - 55	0.5:1	568 ± 70	0.261	6.0±0.5	7213 ± 147	0.136

The PDI values determined for both types of interpolyelectrolyte complexes were within 0.182-0.261, which is typical for moderately polydisperse systems. PEGylated nanoparticles showed better colloidal stability during their storage at 5 °C within 2 months, compared to EPO / L100-55, which partially precipitated after 3 weeks.

Zeta-potential values of nanoparticles are very consistent with the polymer ratios used for their preparation. The polycomplexes formed with excess of cationic EPO have positively charged surface and the nanoparticles formed with 0.5:1 EPO / L100-55 ratio are practically non-charged. Their PEGylated analogues display lower values of zeta-potential. This is related to the screening effects caused by nonionic PEG.

The morphology and structure of these nanoparticles immediately after mixing the polymers in solutions and also after 7 days of storage were additionally evaluated using transmission electron microscopy (TEM, Figure 3).

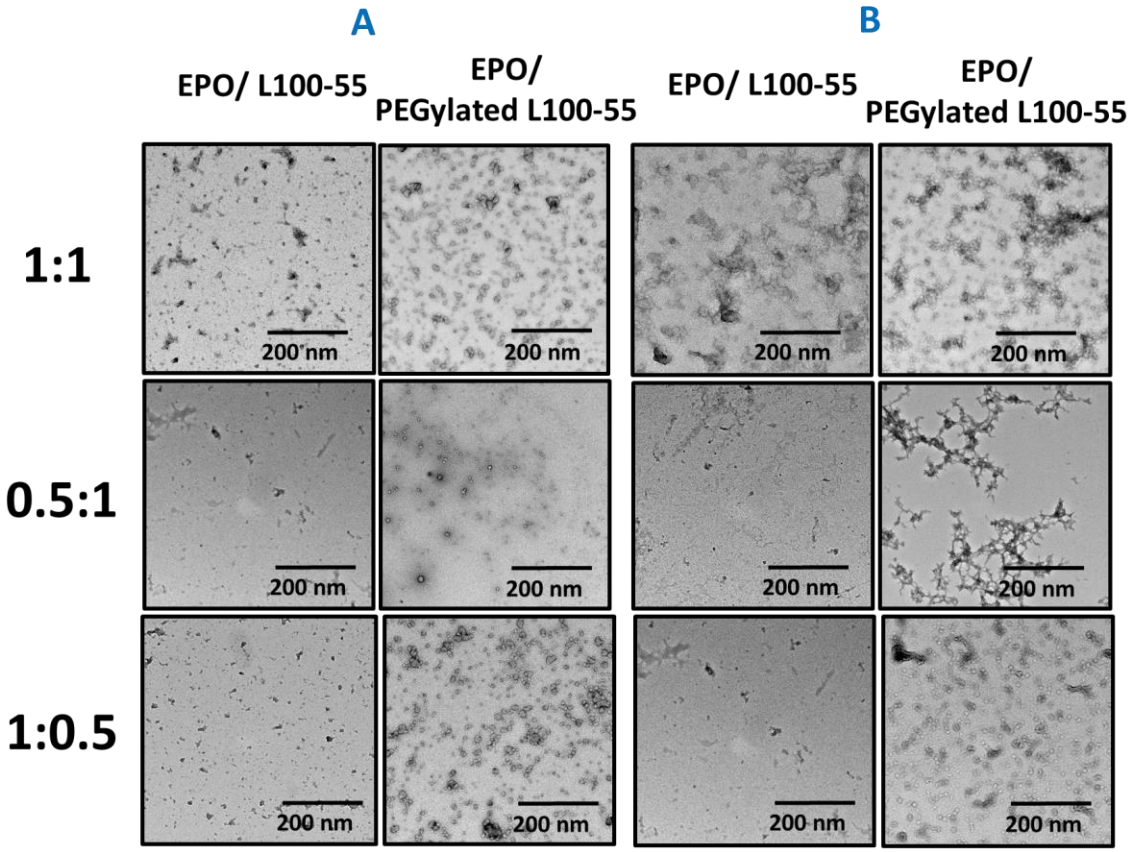


Figure 3. TEM micrographs of EPO / L100-55 and EPO / PEGylated L100-55 at different ratios (by weight) immediately after polymer mixing in solutions (A) and after 7 days of storage at 5 °C (B). Scale bars are 200 nm.

TEM results demonstrate the presence of nanoparticles in both EPO / L100-55 and EPO / PEGylated L100-55. However, the size of all these nanoparticles is < 50 nm, which is substantially smaller compared to the sizes measured using DLS. This discrepancy between TEM and DLS results is well known in the literature and is usually explained by several factors, one of these is likely related to partial dehydration of nanoparticles during sample preparation for TEM.[42] The nanoparticles of EPO / PEGylated L100-55 appear to be less dark, indicating that uranyl ions used as a staining agent did not fully penetrate the nanoparticles core, which indirectly confirms the presence of PEG corona that inhibits this penetration. The images of nanoparticles taken following their 7 days storage show the presence of more pronounced

aggregation and bridging between them. The IPECs prepared at 1:1 ratio of EPO / L100-55 and EPO / PEGylated L100-55 were chosen for further studies as the most colloidally-stable system.

In vitro retention studies

The fluorescently-labelled EPO (EPO_{Fl}) was synthesized by reacting this polymer with 6-iodoacetamidofluorescein as described in Supplementary Information. This derivative was used to prepare fluorescently-labelled nanoparticles.

The retention properties of fluorescently-labelled nanoparticles on freshly excised sheep nasal mucosa were studied using the procedure described previously.[12] Figure 4 shows fluorescent images for the retention of FITC-dextran (negative non-mucoadhesive control), EPO_{Fl}, EPO_{Fl} / L100-55 and EPO_{Fl} / PEGylated L100-55 on freshly excised sheep nasal mucosa irrigated with artificial nasal fluid (ANF). These fluorescent images were analysed using ImageJ software and results are presented in Figure 5.

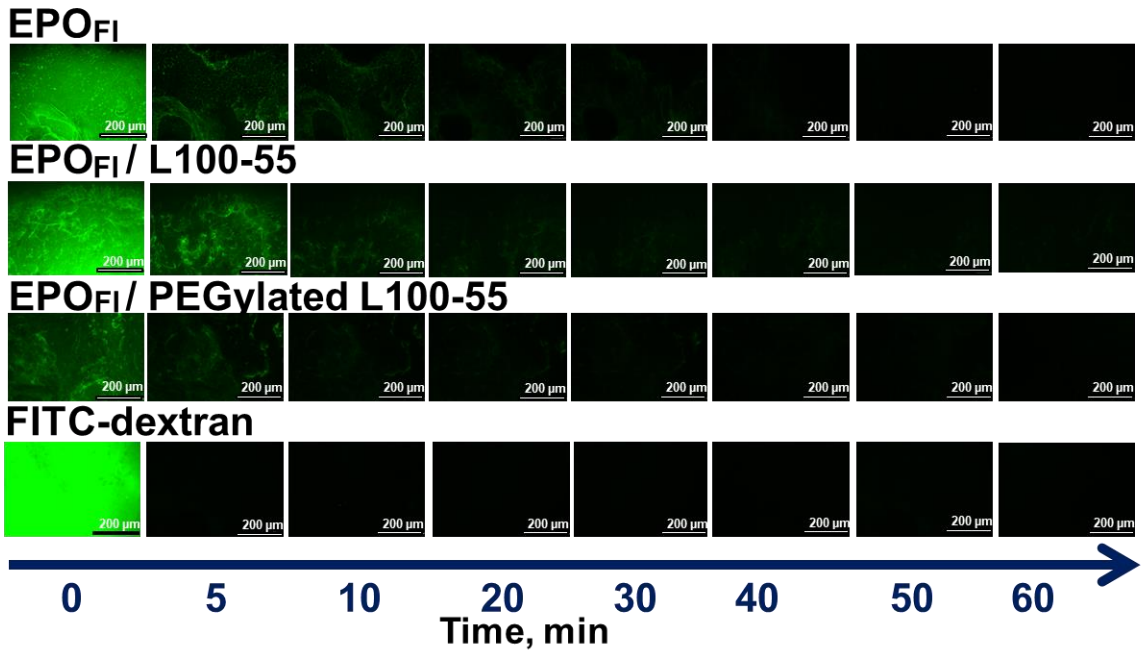


Figure 4. Fluorescent images for retention of EPO_{Fl}, EPO_{Fl} / L100-55, EPO_{Fl} / PEGylated L100-55 and FITC-dextran on sheep nasal mucosa as washed with ANF. Scale bar is 200 µm.

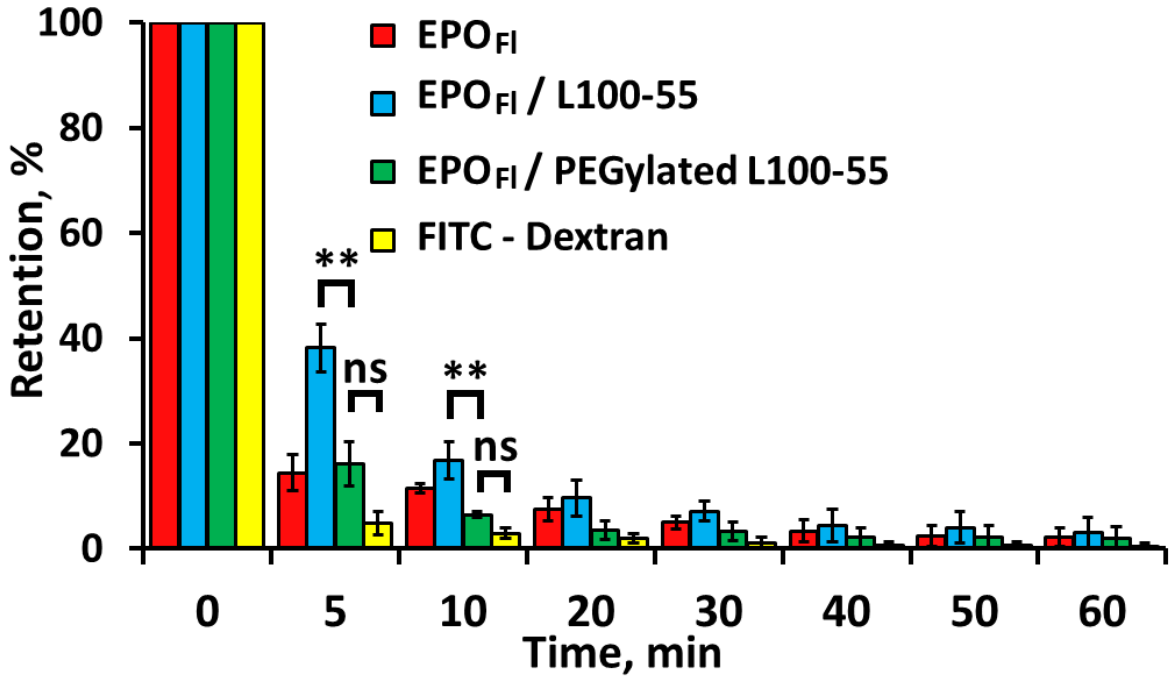


Figure 5. Retention of EPO_{Fl}, EPO_{Fl} / L100-55, EPO_{Fl} / PEGylated L100-55 and FITC-dextran on sheep nasal mucosa as a function of time washed with ANF (n=3, mean ± SD, "*" represents $p < 0.05$).

According to the results, EPO_{Fl}, EPO_{Fl} / L100-55 and EPO_{Fl} / PEGylated L100-55 exhibit greater mucoadhesive properties compared to non-mucoadhesive FITC-dextran. Mucoadhesive properties of EPO_{Fl} are related to its cationic nature and ability to bind to negatively-charged mucosal surface electrostatically. The retention of EPO_{Fl} / L100-55 nanoparticles on the nasal mucosa is greater compared to EPO_{Fl} with approximately 38 ± 5 % of fluorescence was retained on the mucosa after 5 minutes and 17 ± 4 % after 10 minutes. Better retention of interpolyelectrolyte complexes based on EPO / L100-55 compared to pure EPO is possibly related to their insoluble nature and slower elimination from the mucosal surface. A quicker decrease in fluorescence intensity was observed for the retention of EPO_{Fl} / PEGylated L100-55 nanoparticles in contrast with EPO_{Fl} / L100-55: after 5 minutes it showed 16 ± 4 % after 10 minutes it was 6 ± 1 %. This poorer mucoadhesive performance of EPO_{Fl}/PEGylated L100-55 compared to EPO_{Fl} / L100-55 was expected as it is associated with the presence of PEG shell on the surface of nanoparticles. Potentially it reduces the attractive interactions with mucosal surface and makes these nanoparticles less mucoadhesive but more mucus-penetrating.[8,23,34,43,44]

In vivo catalepsy experiments

Haloperidol is a well-known neuroleptic drug that causes catalepsy in laboratory animals.[35,45] Catalepsy is a condition that is characterised by a loss of sensation and consciousness by animals leading to rigidity of their body. Haloperidol-induced catalepsy in

rodents is an established procedure in neuropharmacological studies that allows minimally invasive evaluation of drug penetration to the brain.[46]

Haloperidol-loaded nanoparticles were prepared in the present work based on EPO / L100-55 and EPO / PEGylated L100-55. The mean haloperidol concentrations in the dispersions were 770 ± 6 μg / mL for EPO / L100-55 and 774 ± 8 μg / mL for EPO / PEGylated L100-55 nanoparticles. The encapsulation efficiency of haloperidol into the nanoparticles was around 11-15 %. A commercial formulation of haloperidol (5 mg/mL haloperidol sterile solutions containing lactic acid (Ozone Pharmaceutical Ltd, Russian Federation), diluted to the concentration 770 μg / mL was used as a control.

The catalepsy in rats can be evaluated by measuring the time an animal remains resting on an elevated horizontal bar.[46] Figure 6 shows the study of catalepsy in rats following nasal administration of haloperidol solution (positive control), nanoparticles EPO / L100-55 and EPO / PEGylated L100-55 containing haloperidol. The first appearance of catalepsy is observed in 10 minutes following the nasal administration of all three solutions; however, there is a significant difference in the extent of the effects observed at 10 and 20 min of experiments. Haloperidol incorporated in EPO / PEGylated L100-55 nanoparticles clearly shows more pronounced effect, with the residence time of animals on the rod reaching 178 ± 5 sec at 10 min and 174 ± 5 sec at 20 min of experiments. It is likely that the improved efficiency of this formulation is due the PEGylated corona of the nanoparticles that facilitates their travel across nasal cavity and mucosal penetration to reach olfactory region that is providing direct access for haloperidol molecules to the brain. The administration of haloperidol within more mucoadhesive nanoparticles based on EPO / L100-55 resulted in significantly lower residence time of rats on the rod (59 ± 49 sec at 10 min and 108 ± 61 sec at 20 min) compared to EPO / PEGylated L100-55 nanoparticles. However, this effect is still significantly greater compared to the positive control with free haloperidol.

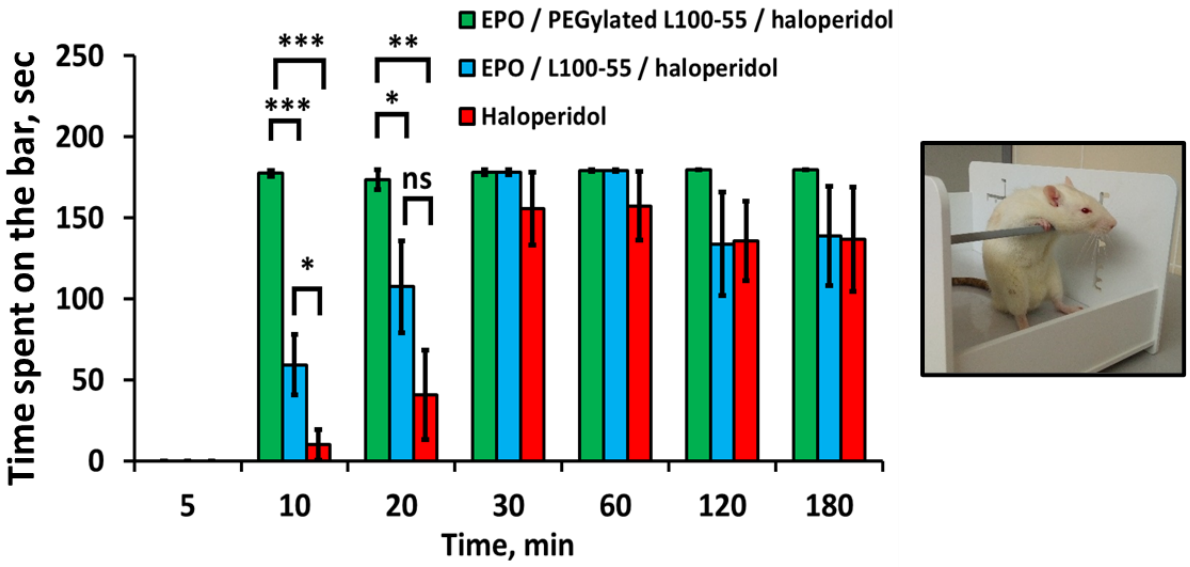


Figure 6. *In vivo* catalepsy test in rats caused by haloperidol (1 mg / kg, nasal), EPO / L100-55 / haloperidol (1 mg / kg, nasal) and EPO / PEGylated L100-55 / haloperidol (1 mg / kg, nasal) (n=6, mean ± SD, "*" represents $p < 0.05$). Inset: exemplar image of rat on elevated rod displaying catalepsy effect.

Discussion

Pioneering research by Nagai and coworkers conducted in the last two decades of the twentieth century has resulted in the first use of water-soluble polymers in the design of mucoadhesive dosage forms for nasal drug delivery.[47,48] The improvement in the drug retention in the nasal cavity using mucoadhesive polymers has been widely exploited and different polymeric systems were used to achieve optimized therapeutic effects.[10] However, the anatomical and physiological features of the nasal cavity impose some limitations regarding the applicability of mucoadhesive dosage forms. The nasal epithelium is a highly dynamic system, characterised by the presence of a mucus blanket that continuously being reformed through the production of mucins and movement as a result of mucociliary clearance.[49,50] This

mucus blanket also acts as a barrier, inhibiting the penetration of not only nanomedicines but also small drug molecules.[51] In this environment a mucoadhesive dosage form could potentially get trapped in the mucus layer and be removed quickly before the active ingredient could be released and reach epithelial cells.

More recent research by Hanes et al.[36] has demonstrated a new concept in transmucosal drug delivery called mucus-penetrating particles. These particles are coated with a mucus-inert short chains of poly(ethylene glycol), which prevent adhesive interactions with mucins and facilitate their diffusion through the mucus. Numerous *in vitro* studies were reported with model PEGylated particles to confirm their ability to diffuse through various mucosal barriers.[8,21,25,36] Some *in vivo* studies also demonstrated the improved efficiency of PEGylated nanoparticles to deliver therapeutic agents via different mucosal membranes.[52,53] However, at the moment the number of *in vivo* studies demonstrating the enhanced therapeutic potential of these nanocarriers is still limited. There is still an open question whether mucus-penetrating particles are more advantageous compared to mucoadhesive particles in nasal drug delivery.

In this work we have utilized intermacromolecular interactions between oppositely charged Eudragits® in the design of nanoparticles that could serve as a nanocarrier for haloperidol. When unmodified Eudragits® were used, the interactions between them resulted in the formation of positively-charged nanoparticles, which clearly exhibited mucoadhesive properties and improved retention on the nasal mucosa.

The presence of reactive carboxylic groups in Eudragit® L100-55 also provided an opportunity for conjugation of this polymer with O-(2-aminoethyl)polyethylene glycol to form PEGylated derivative. When this derivative was involved in intermacromolecular interactions

with Eudragit® EPO the resulting nanoparticles had PEGylated surface and substantially reduced mucoadhesive properties. The absence of mucoadhesive properties in these nanoparticles imparted them enhanced mucus-penetration potential.

A number of optimisation experiments were performed to achieve two types of nanoparticles (mucoadhesive and mucus-penetrating) with comparable physicochemical characteristics and haloperidol load. These nanoparticles were then evaluated *in vivo* in rats for their ability to deliver haloperidol from nose-to-brain. The efficiency of drug delivery was evaluated using a non-invasive catalepsy test, which provides information on the onset and duration of the drug effect. These *in vivo* experiments demonstrated that the formulations can be arranged in the following order by their efficiency of nose-to-brain drug delivery: PEGylated nanoparticles > non-PEGylated nanoparticles > free haloperidol. The mucus-penetrating PEGylated nanoparticles achieved quicker onset of action and potentially greater and longer intensity of the effect of haloperidol.

Our results on nasal dosing of rats with haloperidol formulations are in good agreement with the study of Katare et al,[54] who reported the intranasal administration of this drug formulated using cationic dendrimers. They observed the catalepsy effects at similar intensities but did not look at the onset of action for their formulations.

Conclusions

In the present work, chemical modification of Eudragit® L100-55 through PEGylation was undertaken and the resulting polymer was characterised using several physicochemical techniques.

Two types of interpolyelectrolyte complexes based on EPO / L100-55 and EPO / PEGylated L100-55 were prepared in the form of colloidally-stable nanoparticles. The nanoparticles based on EPO / L100-55 exhibited mucoadhesive properties, whereas EPO / PEGylated L100-55 nanoparticles were substantially less mucoadhesive. Both types of nanoparticles were used to formulate haloperidol and their efficiency to induce catalepsy in rats was evaluated following nasal administration. It was established that administration of haloperidol in non-mucoadhesive EPO / PEGylated L100-55 nanoparticles resulted in significantly more pronounced *in vivo* effects compared to the drug formulated using mucoadhesive EPO / L100-55 nanoparticles.

Acknowledgments

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