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DEVELOPMENT AND CHARACTERIZATION OF *IN SITU* GEL SYSTEM FOR NASAL DELIVERY OF LEVODOPA

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ABSTRACT

The objective of the present study was to develop a thermo sensitive *in situ* gel system based on chitosan (CS) and poly vinyl alcohol (PVA) for nasal delivery of Levodopa. The hydrogel was prepared by mixing the chitosan and poly vinyl alcohol. The concentration of the components was optimized during formulation development. The prepared hydrogel was characterized for gelation temperature, gelation time, viscosity changes, degree of swelling, *in vitro* release and *in vivo* activity. The prepared hydrogel was liquid at room temperature while underwent thermal transition from solution below or at room temperature to non-flowing hydrogel when incubated at 37^{o}c within 12-15 minutes with increased viscosity. The *in vitro* release of Levodopa from gel network was observed, the release of levodopa through gel network decreases upon increasing the chitosan concentration from 1 to 5%. Furthermore, the formulation when evaluated for their *in vivo* activity results indicates that the proposed thermo sensitive *in situ* gelling system has substantial potential as nasal delivery system for Levodopa.

Key words: *In situ* gel system, Nasal delivery, Levodopa, Chitosan, PVA.

INTRODUCTION

Conventionally, the nasal route of delivery has been used for delivery of drugs for treatment of local diseases such as nasal allergy, nasal congestion and nasal infections. Recent years have shown that the nasal route can be exploited for the systemic delivery of drugs such as small molecular weight polar drugs, peptides and proteins that are not easily administered via other routes than by injection, or where a rapid onset of action is required (Illum *et al*., 1998). There are some limitations of nasal route as well like mucosal tissue irritation, rapid mucociliary clearance of the drug from the site resulting in a short duration of time period staying at the site of absorption, low permeability of the nasal membrane for high molecular weight compounds, and various pathological conditions such as cold or allergies which may alter the drug absorption from the nasal cavity.

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bioadhesion to minimize the effect of mucociliary clearance thereby improving absorption. (Leung and Robinson 1990; Duchene and Ponchel 1993).

Recently chitosan has gained wide attention as a polymer for the drug and vaccine delivery. Chitosan [2-amino-2-deoxy-(1→4)-β-D-glucopyranan] is a mucopolysaccharide obtained by the alkaline hydrolysis of chitin, a process which randomly deacetylates and shortens the chain length of the chitin molecules. It is commercially available in a range of grades with different molecular weights and degrees of deacetylation. There are various favourable properties which make the chitosan as a polymer of interest like nontoxicity (Rao and Sharma 1997), biodegradability (Chen and Chen 1998; Muzzarelli 1997), biocompatibility (Hirano and Noishiki 1985; Hirano *et al*., 1988) and mucoadhesiveness (Lehr *et al*., 1992 & Illum *et al*., 1985) demonstrated in animal models, that chitosan solution was able to improve the transnasa1 absorption of insulin. Higher viscosity of the prepared solution and presence of positively charged amino group which bind with negatively charged sialic acid residue on mucous, makes the chitosan mucoadhesive and transient widening of the tight junction makes it a suitable penetration enhancer.

Poly (vinyl alcohol) is a hydrophilic, semisynthetic, copolymer of vinyl acetate and vinyl alcohol and degree of hydrolysis indicates the percentage of vinyl alcohol in the copolymer. PVA system has been explored for use in biomedical applications such as drug delivery devices (Aleyamma *et al*., 1991; Tamura *et al*., 1986), contact lenses (Hyon *et al*., 1994) and artificial organs (Noguchi *et al*., 1991). Biocompatibility of PVA implants has been proved in rabbits (Kodama *et al*., 1996). By changing the degree of hydrolysis it is possible to control the polymers dissolution characteristics in water enabling water soluble as well as insoluble PVA devices. The co polymeric nature of PVA provides the polymer with unique gelling characteristics, which in turn are responsible for its adhesive properties (Korsmeyer *et al*., 1983). Previously chitosan and PVA blend has been used by Minoura et al. (1998) and Koyanoa et al. (2000) to study its surface properties and relationship of these properties with the cell attachment/growth behavior, Zheng et al. (2001) to form fibers, Kim et al. (2002) for electrical charge sensitivity, Wang et al. (2004) for pH sensitivity, Tang et al. (2006) for rheological characterization of the blend.

The objective of the present study was to develop a clear in situ gel system with no or less toxicity for nasal levodopa delivery with a better antiparkinson's effect and thus sustained drug delivery would be expected from such gelling systems. Chitosan and PVA were selected for the formulation development on the basis of earlier studies, reporting their safety and efficacy.

Investigations, results and discussion

Optimization of hydrogel based on thermosensitivity

The formulation was optimized with respect to thermosensitivity and it's characterized by gelation time. The gelation time is defined as the time when the storage modulus become higher than the loss modulus. The gelation time varies from 46.7±4.62 to 30.2±3.04 (min) and 47.9±5.02 to 20±5 (min) from the results obtained in table 1, 2.

It can be concluded that the formulation C and C1 have shown minimum gelation time i.e. 14.4 ± 2.63. It is more desirable because the time taken to convert in gel is in less time, when temperature is elevated, the intermolecular hydrogen bonding interactions are reduced and the energized water molecules surrounding the polymer are removed. The dewatered hydrophobic chitosan chains associate with each other. As a result, a gel is formed and shows better results in thermosensitivity testing.

Now, on the basis of thermosensitivity results the formulation C was selected and it's optimized based on temperature the gelation time varies from 12.9±1.52 to 29.2±2.3 (min). It can be concludes that the chitosan is a cationic polymer having pH-dependent solubility. In acidic solution i.e. below its pKa value (6.2), it gets solubilized and its free amino group ($-NH_2$) gets converted in protonated form ($-NH_3^+$). In acidic solution due to the presence of the $-NH_3^+$ it acts as a weak acid. Due to presence of this charge, electrostatic repulsion will be there and chitosan chains remain separated. The addition of a weak base ($NaHCO_3$) will obviously increase the pH which cause deprotonation ($-NH_3^+$ to $-NH_2$) and at the same time basification can also reduce the electrostatic repulsion between chitosan molecules, which subsequently allows for extensive hydrogen bonding and hydrophobic interactions between chains, and eventually leads to the formation of a white-like precipitate above pH 6.2. In the present study, the precipitates could be prevented by the addition of PVA. Therefore, when the mixture solution of PVA and $NaHCO_3$ is added at low temperature, hydrogen bonds exist not only between the OH group of PVA and the OH and NH_2 groups of chitosan but also between PVA and water due to the high hydrophilicity of PVA, which can lead to the dissolution of chitosan chains figure 1a.

At the same time, the low temperature can also reduce the mobility of chitosan molecules, which further prevents the association of chitosan chains. When temperature is elevated, the intermolecular hydrogen bonding interactions are reduced and the energized water molecules surrounding the polymer are removed. The dewatered hydrophobic chitosan chains associate with each other. As a result, a gel is formed in figure 1b. Therefore, hydrophobic interactions are assumed to be the main driving force to form the gel consisting of chitosan and PVA at high temperature (Tang *et al*., 2007).

Morphology of hydrogels

The cross section of the Chitosan-PVA gel was examined by scanning electron microscopy to investigate morphology and compatibility between Chitosan and PVA molecule (Figure 2). A smooth and homogenous morphology was observed suggesting high miscibility and blend homogeneity between Chitosan and PVA. The good miscibility may sustained by hydrogen bonds and intermolecular interaction between Chitosan and PVA in gel.

Rheological measurement

Rheological studies showed increased viscosity with time after incubating the formulation at 37^{o}C.The viscosity change with time was also measured at 34^{o}C and 40^{o}C, so that effect of various physiological conditions like hyperthermia on viscosity of the formulation can be measured. At the same time point the viscosity of the formulation was different under different temperature conditions and time taken to convert into gel varied with temperature as shown in figure 3.

If hyperthermia cause increased viscosity of the formulation then it may cause obstruction of the nasal cavity which will result in interference with the normal breathing. After converting into gel at various temperature conditions it did not show any difference in viscosity. Hence it can be concluded that in physiological inconsistencies patient will not suffer with obstruction in normal breathing. The formulation also exhibited pseudoplastic character i.e. decrease in viscosity with increase in shear. The formulation showed the pseudoplastic character because due to shearing action the disarranged chitosan molecules align themselves along their long axis in the direction of motion with reduced internal resistance (Kashyap *et al*., 2007).

Degree of swelling

Degree of swelling is to increase in volume or become larger as a result of pressure from within expand or dilate. The percentage of degree of swelling was varied from 6.79 ± 1.01 to 3.14 ± 0.24 (%) in different batches. Degree of swelling studies was conducted in phosphate buffer solution (pH 6.5) maintaining the buffer solution at 37±1°C. The formulations containing different amount of chitosan and PVA was allowed to swell in buffer solution until equilibrium swelling. The maximum degree of swelling (%) was observed in formulation C (CS 3%, PVA 2%). The minimum degree of swelling was observed in formulation A (CS 1%, PVA 4%). Hydrogels are the substances which can absorb water from the nasal mucosa, thus resulting in the temporary dehydration of the epithelial membrane and the opening its tight junctions. (Khafagy *et al*., 2007). The mucoadhesive nature of chitosan and temporary dehydration of the epithelial membrane by hydrogel may cause synergistic effect on the tight junction opening and increased drug absorption through the nasal mucosa. The minimum degree of swelling was observed in formulation A which may be attributed to high concentration of PVA, which results in more compacted gel structure (Tang *et al*., 2007).

If only concentration of PVA is responsible for the gel strength then, maximum degree of swelling should be shown by formulation C (CS 3%, PVA 2%), because due to minimum concentration of the PVA the less compact gel will be formed. The possible explanation for this inconsistency may be that chitosan has pH dependent solubility and at increased pH it shows precipitation. But in the presence of PVA at increased pH it forms gel rather than precipitation. The concentration to prevent precipitation and to make a gel network may be minimum of 2%, so that sufficient free groups $–NH_2$ and –OH on the chitosan molecule may be available to form hydrogen bonds with the water molecules shown in figure 4.

***In vitro* release study**

Release profile data indicates that, the levodopa release from plain levodopa solution was about to 96.5 % while it was in the range of 67-74% by various gel formulations during 12 hours release study. The maximum release was obtained by formulation A, i.e., 74.3±0.85% and minimum release was obtained by the formulation E, i.e., 67.4±0.33% during 12 hours release study. A high release of levodopa from plain levodopa solution was attributed. The release of levodopa through gel network decreased from formulation A to E upon increasing the chitosan concentration from 1 to 5% because of the highly compact gel network and at high temperature the intermolecular hydrogen bonding interactions was reduced and energized water molecules surrounding the polymer are removed. The results are shown in Figure 5.

***In vivo* activity**

Following nasal delivery, the formulation was very quickly absorbed and distributed into the brain (in 30 minutes) as compared to oral route (30 minutes) as shown in figure 6.1, 6.2.

The result suggested that proposed system can be used as a potential nasal drug delivery system and as a good adjuvant therapy for parkinson's patients who experience symptomatic fluctuations by l-dopa oral administration. The chitosan-PVA based gel showed a prolonged antiparkinson's effect and maintained their integrity over a period of time.

The formulation from the nasal cavity is usually cleared by the ciliary movement, but chitosan-PVA based hydrogel system can maintain the levodopa concentration for a longer time (up to 6hr) because of the superior bioadhesivity characteristics of gel. The developed gel formulation prolongs the contact between the formulation and the absorption site in the nasal cavity. In addition, it also promotes direct absorption of the drug through the nasal mucosa.

Chitosan has been shown to possess mucoadhesive properties (Shimoda *et al*., 2001; Kockisch *et al*., 2003) due to molecular attractive forces formed by electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces. These properties may be attributed to:

- Strong hydrogen bonding groups like –OH,–COOH
- Strong charges
- High molecular weight
- Sufficient chain flexibility
- Surface energy properties favoring spreading into mucus

Chitosan possesses no toxicity and can be applied onto the nasal epithelium. It swells and forms a gel like layer in aqueous environment (by absorbing water from the mucous layer in the nasal cavity), which is favorable for interpenetration of polymer and glycoprotein chains into mucous (Felt *et al*., 1998).

The positive charge on chitosan polymer ($–NH_3^+$) gives rise to strong electrostatic interaction with mucus or negatively charged sialic acid residues on the mucosal surface. Chitosan also shows good bioadhesive characteristics and can reduce the rate of clearance of drug from the nasal cavity thereby increasing the bioavailability of drugs incorporated in it. Membranes prepared from chitosan have shown greater permeability for acidic drugs than basic drugs (Sawayanagi *et al*., 1982b).

Chitosan blended with PVA have already been reported to have good mechanical properties (Koyanoa *et al*., 2000) because of the specific intermolecular interactions between PVA and chitosan in the blends. Thus these systems have advantages of ease of administration in solution form and long residence time at the site of absorption due to conversion into gel thus sustained drug delivery would be expected from such gelling systems.

Experimental

Materials

Chitosan was purchased from Fluka Co. Ltd., Switzerland. The degree of deacetylation was 85% Poly (vinyl alcohol), MW 30,000-70,000 Da Sigma Chem. Co. (St. Louis, Mo, USA). The Levodopa, Cipla pharmaceutical, Bombay.

Preparation of hydrogel

The hydrogel was prepared by the method as reported by Tang *et al*., 2007 with slight modifications. A clear solution of chitosan was prepared in 0.1M HCl and 100 mg of drug was added to this solution and the solution was chilled on ice bath for 15 min. PVA was dissolved in preheated distilled water (80^oC). This solution was also chilled on ice bath. Both the solutions were mixed under magnetic stirring for 10 min and the final pH of solution was adjusted to with 1M $NaHCO_3$.

Characterization of thermosensitivity

The process of hydrogel formulation was optimized by increasing the concentration of chitosan and keeping the concentration of PVA constant with respect to thermosensitivity and it's characterized by gelation time. The gelation time was determined at 37±1°C by test tube inverting method (Wu *et al*., 2006). 2.0 ml formulation was added into a tube (10 ml) having diameter of 1.0 cm and kept in a water bath at 37±1°C. The tube was taken out after every 1 min and inverted to observe the state of the formulation. The gelation point was determined by flow or non-flow criterion.

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Rheological measurement

To measure rheological properties change in viscosity with time was measured by Brookfield Viscometer (Model RVDVE 330, Brookfield Eng. Lab. USA) by putting the formulation in a water bath to maintain the temperature that mimic the body temperature.

Degree of swelling

Degree of swelling of the prepared gel was carried out in phosphate buffer pH 6.5. In this, gel of fixed weight corresponding to 10 ml of sol was taken in buffer solution in a tarred petri dish and the gel was allowed to swell until equilibrium swelling reached (complete saturation) with buffer solution. Swelling degree was measured by dividing the weight gained by the original weight.

***In-vitro* release study**

A 10 ml of sol was filled in dialysis bag this was suspended in release media (phosphate buffer (PB) pH 6.5, 100 ml). Release media were maintained at 37±1°C with magnetic stirring at 70 rpm. Sampling was done by withdrawing 2ml of the media at different time points for 12 hours and replaced with fresh media so that volume of the release media remains constant. The samples were analyzed using UV-Visible spectrophotometer 1700 (Shimadzu corporation) at 282nm.

In vitro release of the levodopa from various formulations was investigated using dialysis bag. Dialysis bag was selected for in vitro release study because dialysis bag mimic the behavior of biological membrane. Comparative release was measured between plain levodopa solution and various formulations.

***In-Vivo* activity**

In vivo efficacy of formulation was measured in adult male Wistar rat (weight 200-250 gm). All the animals were housed at the animal laboratory facility provided by Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal. They were maintained under standard environmental conditions and housed individually in plastic cages in a controlled environment (22–24 ^{o}C and 12:12 light–dark cycle) with free access to chow and water. All the experimental protocols were approved by Institutional Animal Ethics Committee, VNS Institute of Pharmacy, Bhopal, India (Elsayed *et al*, 2009).

The formulation (marker) was dissolved in phosphate buffer (pH 6.5) solution and administered unilaterally to the right nostril (10µl) using polyethylene tubes (PE-10) attached to micropipette and different formulation were administered to different group of animals as described in table 4.1. After 30mins the animals were scanned for 3 min using gamma camera (Siemens, Germany) (Dahlin *et al*., 2000). Rats were used to evaluate the pharmacological action of formulation (marker). The total duration of the experiment was 1 h. Rats were divided into 2 groups (n = 3) group A and group B.

Fig 1. Formation mechanism of chitosan/PVA gel (a) solution at low temperature; (b) gel at high temperature

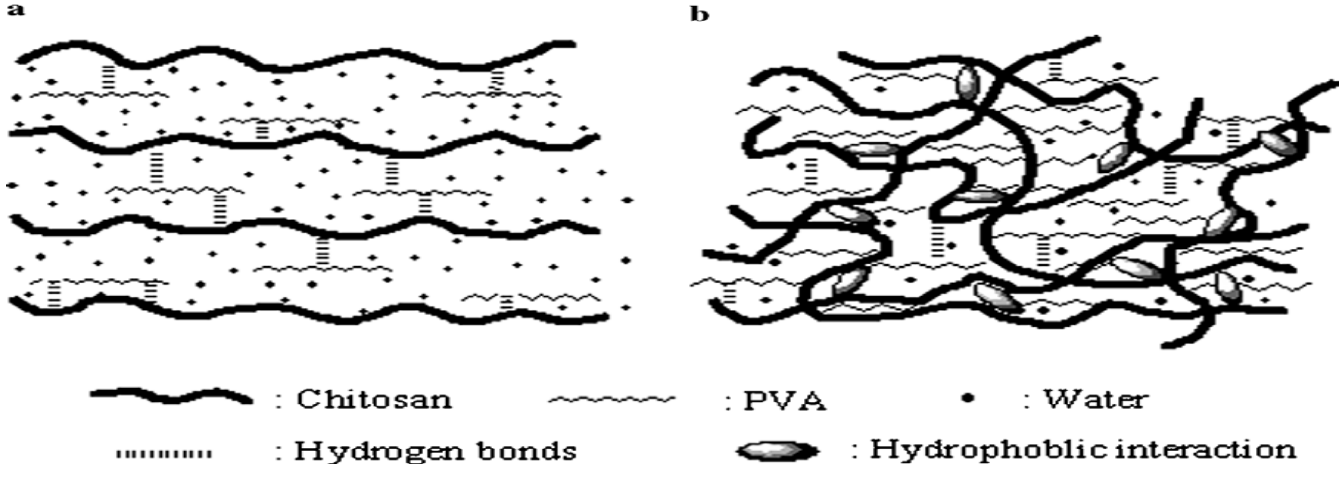


Fig. 2. SEM photograph of chitosan-PVA gel

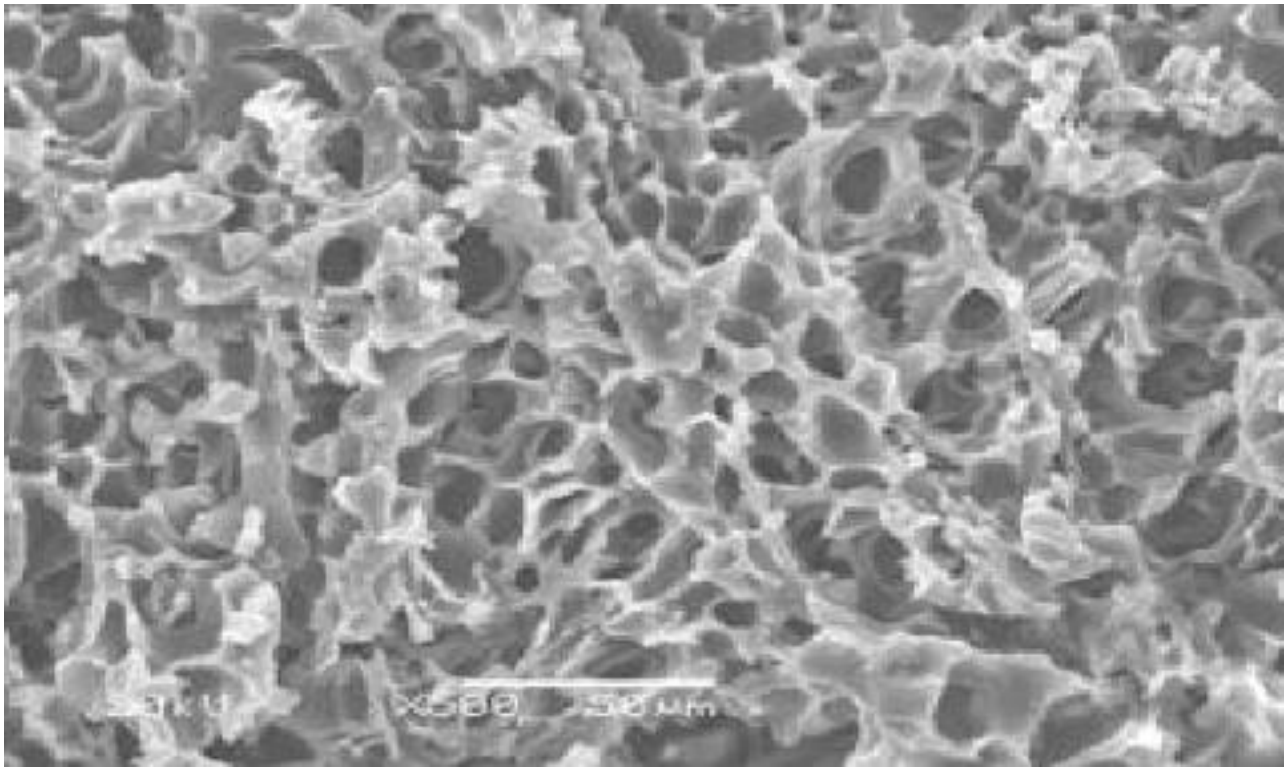


Fig 3. Effect of temperature on gelation time and viscosity of the optimized

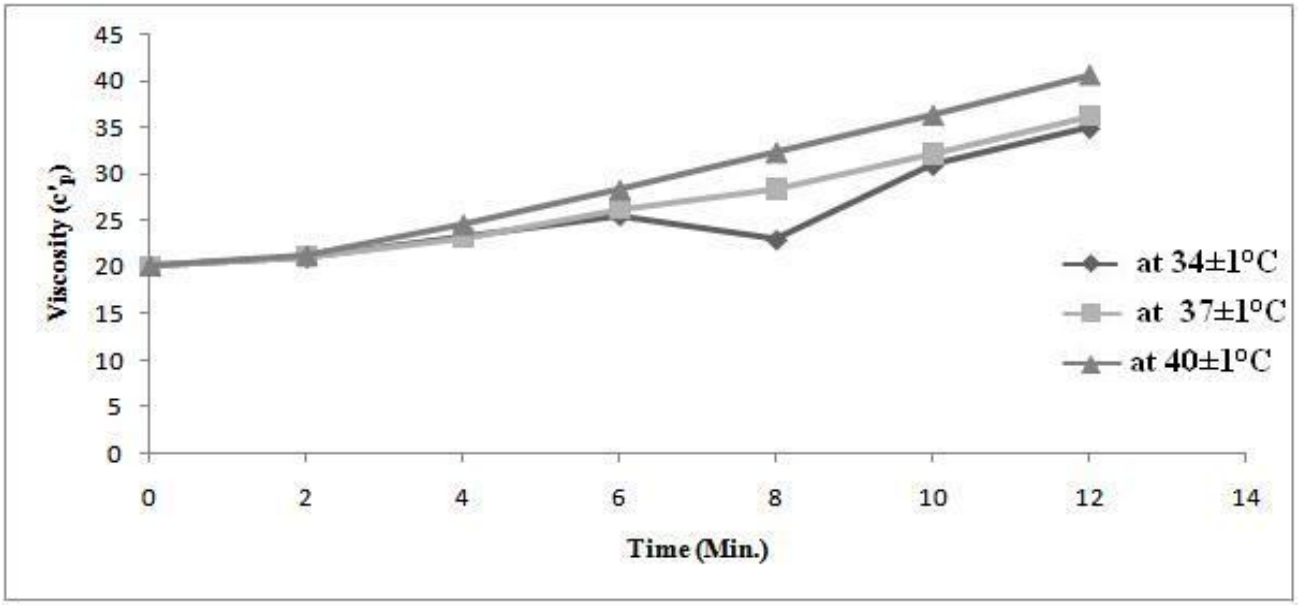


Fig 4. Degree of swelling (%) of various formulations in phosphate buffer solution maintained at 37±1°C

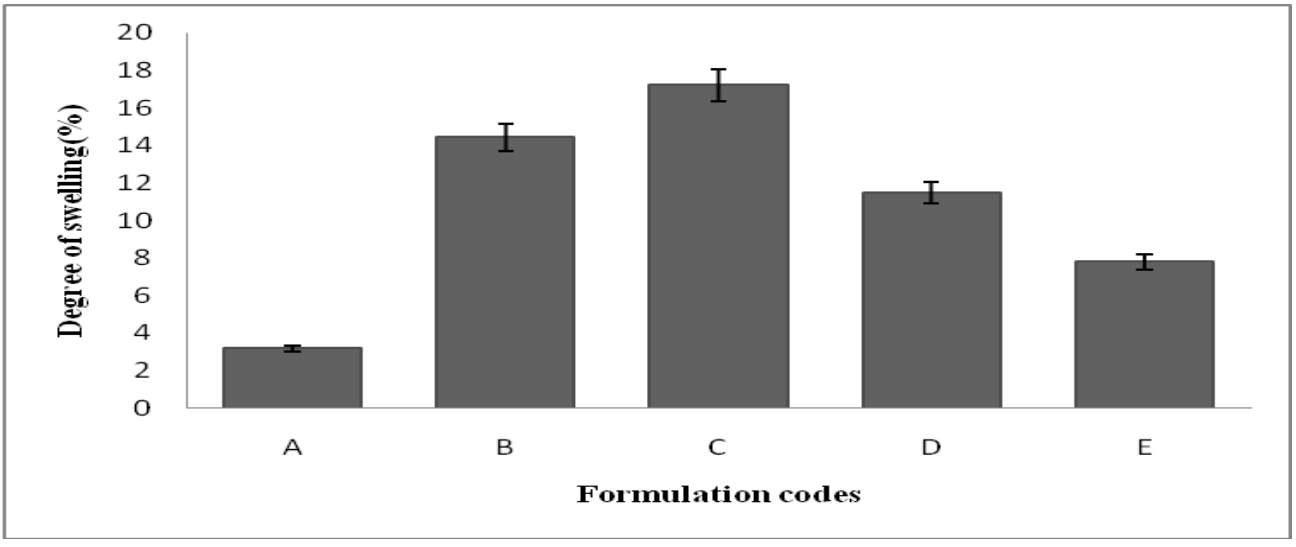


Fig. 5. Cumulative release (%) from various gel formulations in phosphate buffer (pH 6.5) at 37±1°C

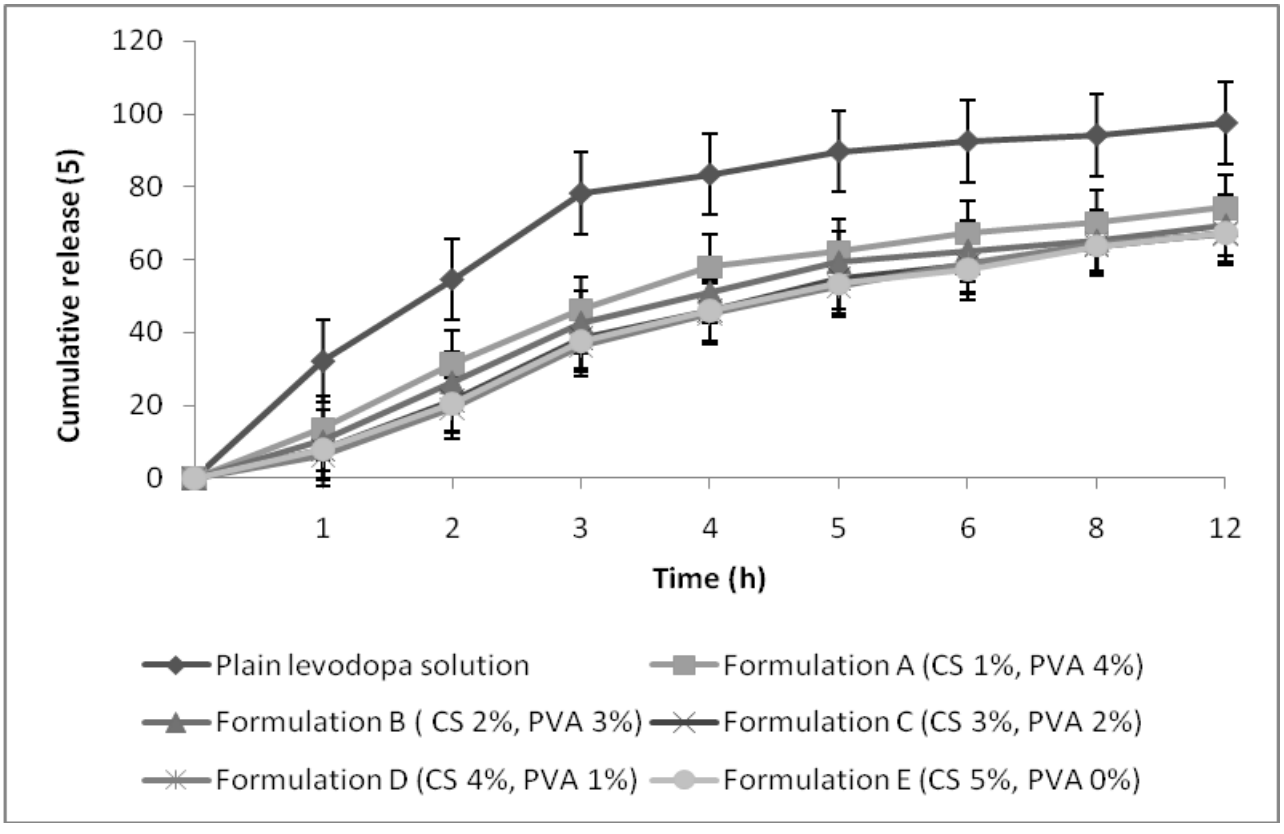
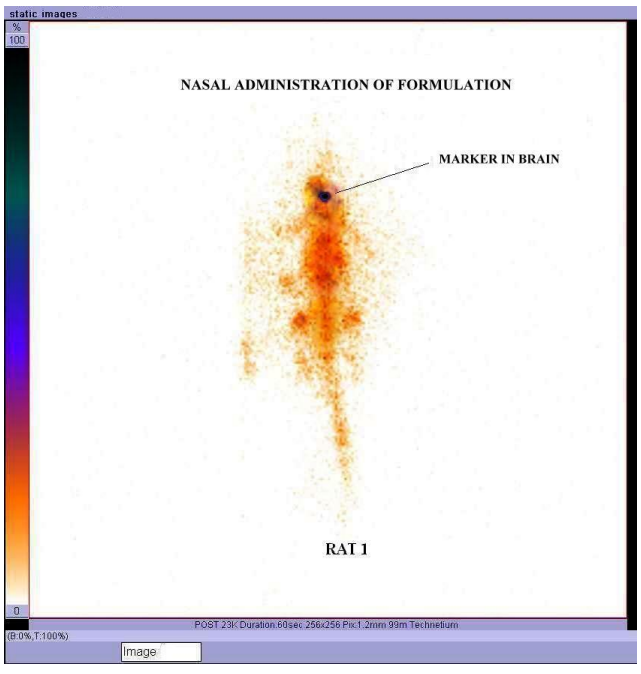
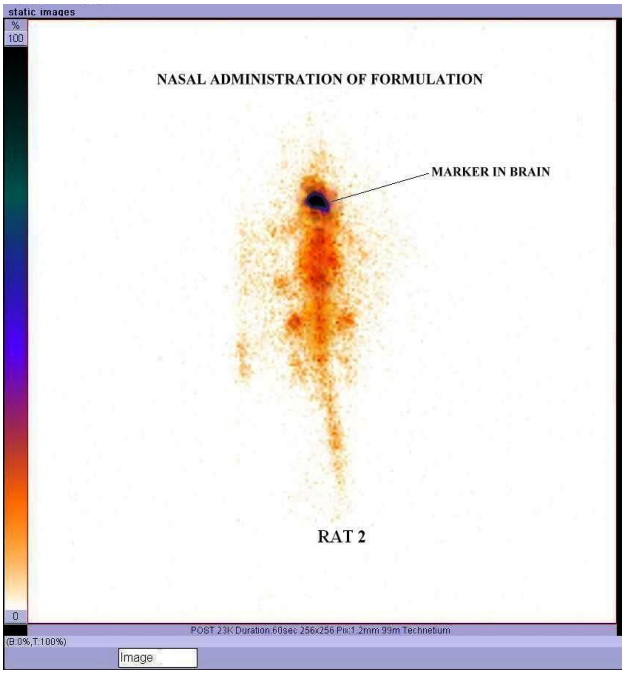


Fig 6.1. Nasal administration of formulation (after 30 minutes)





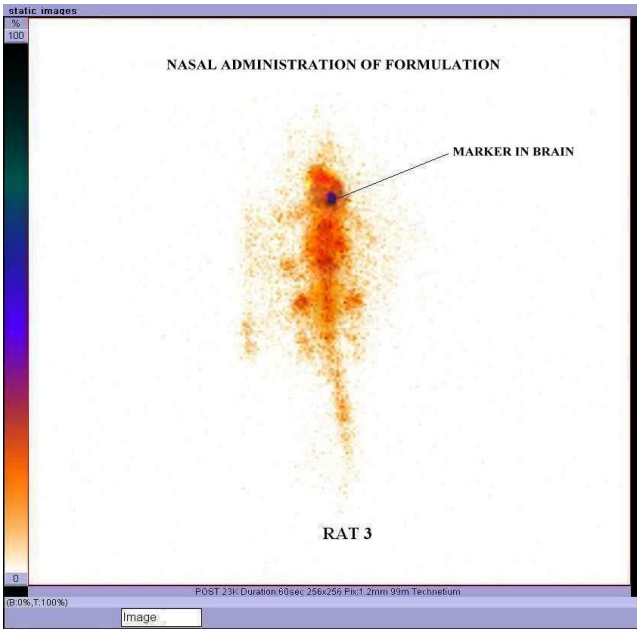
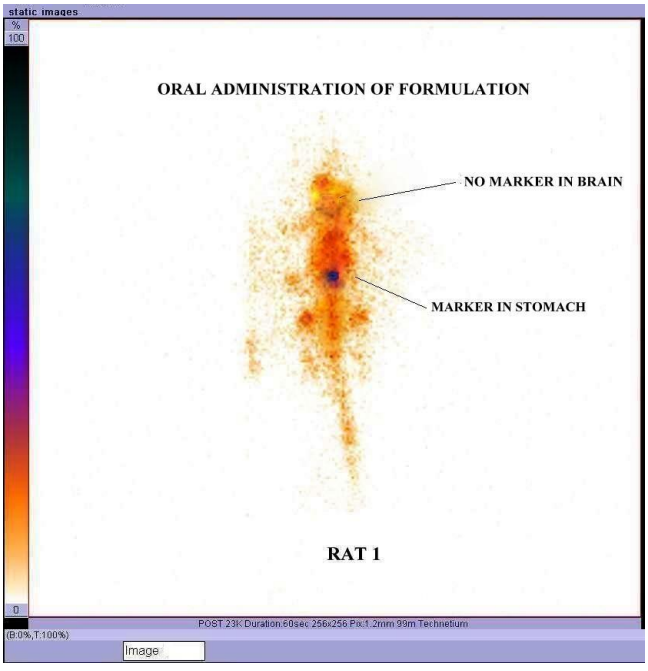
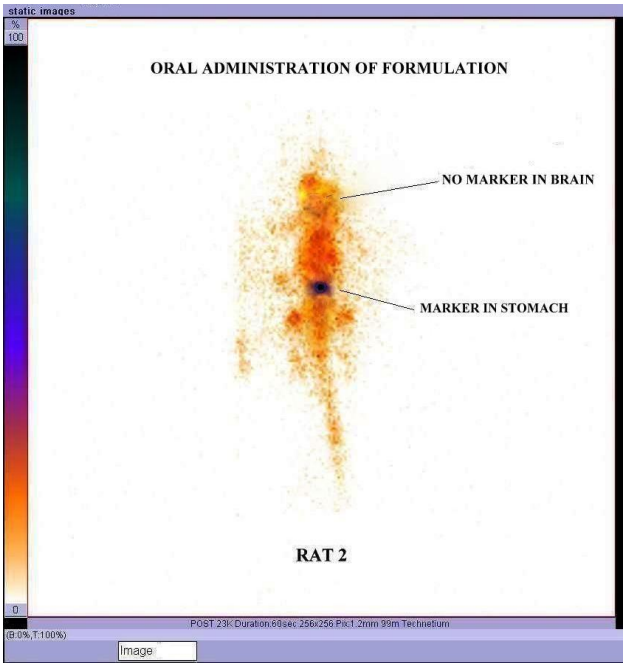


Fig 6.2. Oral administration of formulation (after 30 minutes)





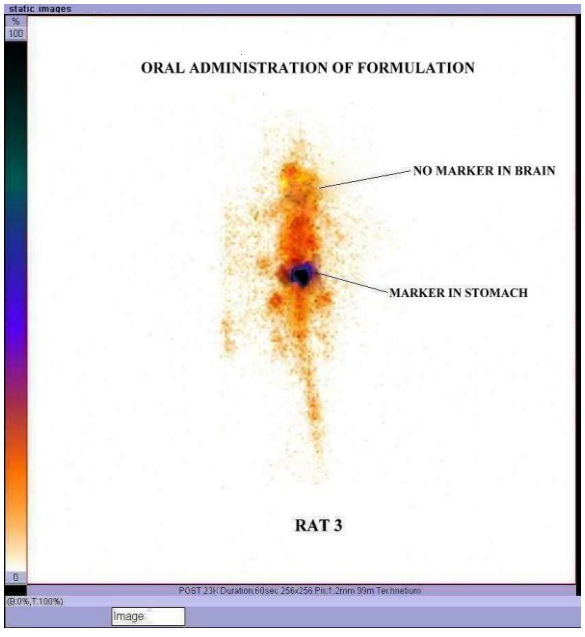


Table 1. Optimization of formulation based on Chitosan concentration

S.No.	Formulation	Chitosan (%w/v)	PVA (%w/v)	Parameter optimized (Thermosensitivity) Gelation time(min.)
1.	A	1	2	30.2 ± 3.04
2.	B	2	2	20 ± 4
3.	C	3	2	14.4 ± 2.63
4.	D	4	2	24.8 ± 3.62
5.	E	5	2	46.7 ± 4.62

(Avg. ± SD)
(n=3)

Table 2. Optimization of formulation based on PVA concentration

S.No.	Formulation	Chitosan (%w/v)	PVA (%w/v)	Parameter optimized (Thermosensitivity) Gelation time(min.)
1.	A1	3	0.5	20 ± 5
2.	B1	3	1	25.9 ± 4.63
3.	C1	3	2	14.4 ± 2.63
4.	D1	3	3	32.6 ± 5.63
5.	E1	3	4	47.9 ± 5.02

(Avg. ± SD)
(n=3)

Conclusion

Chitosan has been proved for its efficacy and safety and due to its permeation enhancing effect by widening the tight junction. It is a polymer of interest especially for hydrophilic compounds of high molecular weight. In the present study thermosensitive formulation was developed by using chitosan and PVA combination which transform from free flowing sol form to non flowing gel form at body temperature. Due to free flowing nature the formulation can be dropped or sprayed easily which convert in to non flowing form at the site of action which prolong the contact time of the formulation at the site of absorption and at the same time sustain the drug release from the gel network. Finally on the basis of study data, the formulation can be suggested as a potential alternative to invasive delivery of levodopa.

REFERENCE

Aleyamma AJ, Sharma CP. Poly (vinyl alcohol) as a biomaterial. In: Sharma CP, Szycher (eds.) Blood compatible material and devices: perspectives towards the 21st century. Technomic. Lancaster PA, 1991, 123-130.

Chen R, Chen HC. Chitin enzymology-chitinase. *Adv Chitin Sci*., 3, 1998, 16-23.

Chien YW, Su KSE, Chang SF. Anatomy and physiology of the nose. In: Chein YW (ed.) Nasal Systemic Drug Delivery, Marcel Dekker Inc., New York, 1989, 1-26.

Felt O, Buri P, Gurny R. Chitosan: a unique polysaccharide for drug delivery. *Drug Dev. Ind. Pharm*., 24, 1998, 979-993.

Hirano S, Noishiki Y. The blood compatibility of chitosan and N-acylchitosans. *J Biomed Mater Res*, 19, 1985, 413-417.

Hirano S, Seino H, Akiyama Y, Nonaka I. Biocompatibility of chitosan by oral and intravenous administrations. *Polym Eng Sci*, 59, 1988, 897-901.

Hyon SH, Cha WI, Ikada Y, Kita M, Ogura Y, Honda Y. Poly (vinyl alcohol) as a soft contact lens material. *J Biomater Sci Polym Ed*, 5, 1994, 397-406.

Illum L. Drug delivery systems for nasal application. In: Hıncal A.A., Kas H.S., Sumnu M, editors. Third International Pharmaceutical Technology Symposium [Proceedings]. Ankara: Meteksan, 1985.

Kashyap N, Viswanad B, Sharma G, Bhardwaj V, Ramaraob p, Ravi Kumar MNV. Design and evaluation of biodegradable, biosensitive in situ gelling system for pulsatile delivery of insulin. *Biomaterials*, 28, 2007, 2051-2060.

Kim S J, Park SJ, Kim IY, Shin MS, K SI. Electric stimuli responses to poly (vinyl alcohol)/chitosan interpenetrating polymer network hydrogel in NaCl Solutions. *J Appl* Polymer *Sci*, 86,2002, 2285-2289.

Kockisch S, Rees GD, Young SA, Tsibouklis J, Smart JD. Polymeric microspheres for drug delivery to the oral cavity: an in vitro evaluation of mucoadhesive potential. *J. Pharm. Sci*., 92, 2003, 1614-1623.

Korsmeyer RW, Gurny R, Doclker E, Buni P, peppas NA. Mechanisms of solute release from porous hydrophilic polymers. *Int J Pharm*, 15, 1983, 25-35.

Koyanoa T, Koshizakib N, Umeharab H, Nagurac M, Minourab N. Surface states of PVA/chitosan blended hydrogels. *Polymer*, 41, 2000, 4461-4465.

Lee WA, Longenecker JP. Intranasal delivery of proteins and peptides. *BioPharm*, 1, 1988, 30-37.

Lehr CM, Bouwstra JA, Schacht EH. *In vitro* evaluation of mucoadhesive properties of chitosan and some other natural polymers. *Int J Pharm*, 78, 1992, 43-48.

Leung SHS, Robinson JR. Bioadhesives in drug delivery. *Polymer News*, 15, 1990, 333-342.

Minoura N, Koyano T, Koshizaki N, Umehara H, Nagura M, Kobayashi K. Preparation, properties, and cell attachment/ growth behavior of PVA/chitosan-blended hydrogel. *Mater Sci Eng*, C6, 1998, 275-280.

Muzzarelli RAA. Human enzymatic activities related to the therapeutic administration of chitin derivatives. *Cell Mol Life Sci*, 53, 1997, 131-140.

Noguchi T, Yamamuro J, Oka M, Kumar P, Kotoma Y, Hyon SH, Ikada Y. Poly (vinyl alcohol) hydrogel as an artificial articular cartilage: evaluation of biocompatibility. *J Appl Biomater*, 2, 1991, 101-107.

Rao SB, Sharma CP. Use of chitosan as a biomaterial: studies on its safety and hemostatic potential. *J Biomed Mater Res*, 34, 1997, 21-28.

Shimoda J, Onishi H, Machida Y. Bioadhesive characteristics of chitosan microspheres to the mucosa of rat small intestine. *Drug Dev Ind. Pharm*, 27, 2001, 567-576.

Tamura K, Ike O, Hitomi S, Isobe J, Shimizu Y, Nambu M. A new hydrogel and its medical application. Trans Am Soc Artif Organs, 32, 1986, 605-608.

Tang YF, Du YM, Hu XW, Shi XW, Kennedy JF. Rheological characterisation of a novel thermosensitive chitosan/poly (vinyl alcohol) blend hydrogel. *Carbohydr* Polymer, 67, 2007, 491-499.

Wang T, Turhan M, Gunasekaran S. Selected properties of pH-sensitive, biodegradable chitosan–poly (vinyl alcohol) hydrogel. Polymer Int, 53, 2004, 911-918.

Wu J, Su ZG, Ma GH. A thermo- and pH-sensitive hydrogel composed ofquaternized chitosan/glycerophosphate. *Int J Pharm*, 315, 2006, 1-11.

Zheng H, Du YM, Yu JH, Huang RH, Zhang LN. Preparation and characterization of chitosan/poly (vinyl alcohol) blend fibers. *J Appl* Polymer *Sci*, 80, 2001, 2558-2565.