Pharmaceutics Research Laboratory, Department of Pharmaceutical Sciences, Doctor Harisingh Gour Vishwavidyalaya, Sagar, India

Pelleted bioadhesive polymeric nanoparticles for buccal delivery of insulin: preparation and characterization

P. VENUGOPALAN, A. SAPRE, N. VENKATESAN and S. P. VYAS

The study was an attempt to develop an alternative buccal delivery system for insulin. Insulin bearing nanoparticles were prepared by the emulsion internal phase evaporation method. The effect of some formulation variables viz., polymer/drug ratio and emulsifier concentration was studied on particle size and entrapment efficiency. Nanoparticles were pelleted to impart three-dimensional structural conformity and coherence thereby facilitating buccal application. Solid lateral and horizontal sedimentaton in the pellet can be avoided by nanoparticulation and ensuring uniform drug distribution throughout the pellet. The *in vitro* studies of the pellets included bioadhesion and drug release profile. *In vivo* studies were performed on diabetic rats. A significant hypoglycemic response was observed after 7 h, without any detectable fluctuation in blood glucose profile and risk of hypoglycemia.

1. Introduction

The buccal mucosa has been investigated extensively as a potential site for delivery of peptides and proteins. The buccal route besides being convenient, accessible and robust, is highly vascularized and hence permits rapid absorption. Additionally it suffers lesser onslaughts by enzymes. It also permits incorporation of locally acting agents such as penetration enhancers, enzyme inhibitors and mucus production suppressing agents. The peptides studied for administration through this route include insulin [1–8], protirelin [9–12], oxytocin [13–15] and vasopressin analogues [16]. In the recent years bioadhesive polymers/copolymers have attracted much attention in the design of delivery systems, oral mucosal routes in particular [17–20]. A bioadhesive delivery system is known to enhance the drug bioavailability by prolonging residence time at the specified regions and an optimal contact with the absorbing biological membrane. Thus, buccal bioadhesive systems additionally permit sustained drug release as long as the system remains adherent to the mucosal membrane. They facilitate high drug concentration in the local area and may even increase the total permeability of protein/peptide moieties.

The present work is an effort to develop buccal delivery system for insulin. In this study, insulin was encapsulated into poly(acrylamide) nanoparticles. Poly(acrylamide) is reported to have excellent bioadhesive characteristics. Pelleting of nanoparticles was performed as it offers three-dimensional structural conformity for acceptable buccal administration. On the other hand nanoparticle formation ensures uniform drug distribution throughout the pellet by avoiding solid lateral and horizontal sedimentation. Furthermore, in the event of pellet disintegration, the discrete particulate may remain adhered to the buccal mucosa. Thus the system offers maximum mucosal retention time. Poly(acrylamide) was selected on account of its well-established bioadhesive, biodegradable, biocompatible and stable nature.

2. Investigations, results and discussion

The insulin bearing nanoparticles were prepared using the emulsion solvent evaporation method. The effect of some independent process variables viz., drug/polymer ratio and emulsifier concentration on the particle size and entrapment efficiency of nanoparticles and on the duration of bioadhesion of the pellets was studied.

Table 1: Effect of drug/polymer ratio on entrapment efficiency and particle size

Formulation code	Insulin weight (mg)	Ratio Insulin/PA	Insulin entrapment (% w/w)	Entrapment efficiency (%)	Particle size (nm)
P1	10	1 : 20	1.9	40.5	820 ± 12
P2	20	1 : 10	5.6	62.4	805 ± 8
P3	30	1 : 7	7.9	63.6	740 ± 18
P4	40	1 : 5	11.7	71.5	684 ± 14
P5	50	1 : 2	16.5	66.1	603 ± 11

It was observed that with an increase in drug/polymer ratio achieved by increasing the amount of insulin dissolved in internal aqueous phase, there was a small decrease in the mean particle size (Table 1). The decrease in particle size with decreased polymer concentration can be attributed to a decreased viscosity of the internal phase. Thus with lesser viscosity, sub-division of polymer into smaller particles became relatively easy leading to stabilization of particle(s) of smaller size. With an increase in the drug/polymer ratio, the entrapment efficiency of the nanoparticles was improved reaching a maximum with a ratio of 1 : 5 (Table 1). However there was no obvious relationship between drug/polymer ratio and entrapment efficiency.

At low concentration of Span 80 (0.5% v/v) the mean particle size was >800 nm. As emulsifier concentration increased, a significant decrease in particle size was evident (Table 2). At an emulsifier concentration of 1.0% v/v, most of the particles were in the size range of 670–690 nm. The increment in the amount of emulsifier led to a de-

Table 2: The effect of emulsifier concentration on entrapment efficiency and particle size

Formulation code	Emulsifier concentration	Insulin entrapment (% w/w)	Entrapment efficiency (%)	Particle size (nm)
P6	0.5	5.1	56.7	831 ± 12
P7	1.0	5.5	61.1	680 ± 9
P8	1.5	5.8	64.4	604 ± 19
P9	2.0	6.0	66.7	581 ± 10

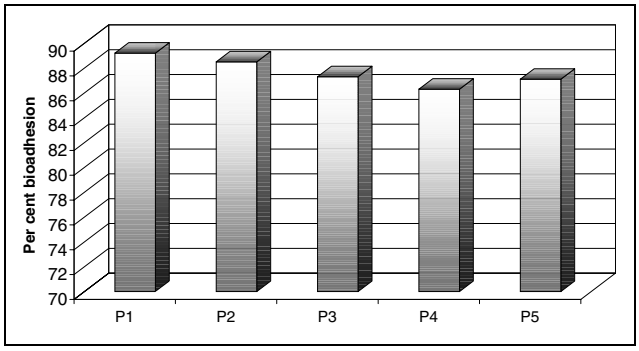

Fig. 1: Per cent bioadhesion of various nanoparticulate formulations

crease in interfacial tension, thereby assisting small droplet fragmentation and also preventing droplet aggregation/coalescence. The entrapment efficiency was improved, but the increase was more or less insignificant.

To check the structural integrity of the insulin after the entrapment procedure, polyacrylamide gel electrophoretic analysis of native insulin and insulin released from nanoparticles was undertaken. No additional bands were observed indicating that the incorporation process did not affect the structural integrity of the insulin significantly.

A bioadhesion study of the polymeric nanoparticles was performed on rabbit intestine to establish qualitative, however relative bioadhesive profile of the system. These studies revealed that the poly(acrylamide) nanoparticles exhibited significant bioadhesion (Fig. 1).

In vitro release studies were performed with pelleted nanoparticles to record the cumulative amount of drug released in simulated saliva solution (Fig. 2). It is apparent from the plot that a sustained drug release profile was achieved and governed by the polymer concentration. With an increase in the polymer content a corresponding decrease in the drug release rate was evident.

In vivo studies were carried out on rats. The method was based on hypoglycemic response to insulin. The fasting blood glucose of diabetic rats was 300 ± 10 mg/dl, as an initial blood glucose concentration. Blood glucose level decreased progressively, after the i.m. Insulin dose (1.4 I.U.) administered and attained 15% of initial blood glucose level at 5 h. To the anesthetized diabetic rats, the pellets containing (1.4 I.U.) insulin were administered through buccal route. A significant hypoglycemic response was observed (Fig. 3). With the formulations nearly 45% initial blood glucose concentration was attained at 7 h, without any fluctuation in blood glucose level.

This study showed that the system exhibits promising results. It was observed that insulin was available for systemic circulation at a controlled rate, without any danger of hypoglycemia, indicating that after optimization of process variables it is possible to develop a bioadhesive system that could effectively maintain normoglycemic condition.

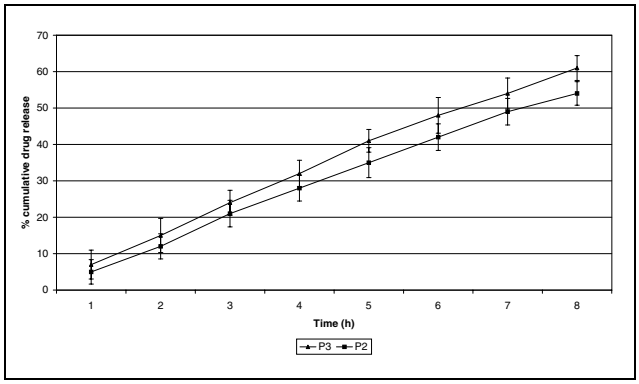

Fig. 2: Per cent cumulative drug release profile of pelleted nanoparticles

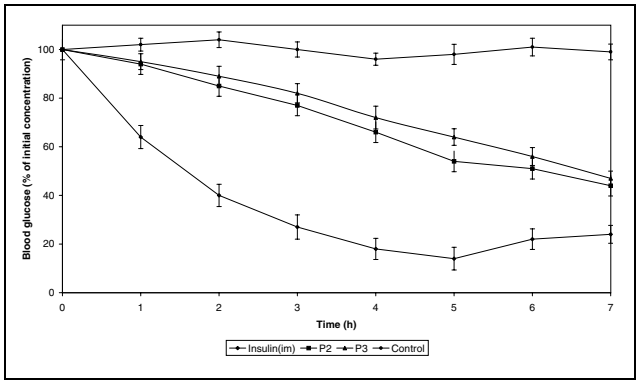

Fig. 3: Blood glucose profile following buccal application of pelleted insulin nanoparticles

3. Experimental

3.1. Materials

Insulin (M.J. Pharmaceuticals), Poly(acrylamide) (Aldrich, Average molecular weight 10,000), Span 80 (Robert Johnson). All other chemicals were obtained from commercial sources and were of analytical grade.

3.2. Preparation of the nanoparticles and pellets

Insulin nanoparticles were prepared by the emulsion internal phase evaporation method. Poly(acrylamide) and insulin were dissolved in acidified methanol (pH 3.0, 5.0 ml). This polar phase was emulsified by sonication (Soniweld, India) at 50 W with light paraffin oil (95.0 ml) containing Span 80 (1% v/v). The so formed w/o emulsion was stirred continuously at room temperature for complete evaporation of the internal phase. The dispersion was passed through a Millipore filter (0.82 μm) to eliminate particles above 1 μm in size. The filtrate was again filtered through a Millipore filter (0.4 μm). The nanoparticles retained over the filter were washed with ether and dried at room temperature. A series of nanoparticles were prepared by varying the polymer:drug ratio, and emulsifier concentration. The nanoparticles were compressed into pellets. The final bioadhesive buccal pellet was a disc of 1 cm^2.

3.3. Characterization

3.3.1. Size and shape

The dried nanoparticles were sized by laser diffractrometry (Malvern 2600 D Laser Sizer). The shape and surface characteristics were examined by scanning electron microscopy (PSEM 515 Philips Holland).

3.3.2. Drug content determination

The drug content determination was carried out with nanoparticles. Nanoparticles (200 mg) were placed in a series of stoppered glass tubes. To each tube methylene chloride (5 ml) was added. The tubes were closed tightly and shaken to dissolve the polymeric mass. 10 ml simulated saliva solution (composition: potassium chloride, 0.4 g; sodium chloride, 0.4 g; sodium sulfide, 0.016 g; magnesium pyrophosphate, 0.016 g; calcium chloride, 0.6 g; disodium hydrogen phosphate, 0.6 g and mucin, 2.0 g in 1 l purified water) was added to each of the tubes and shaken. Aqueous phase containing drug was separated [21]. The organic phase was extracted three times with simulated saliva solution. The separated aqueous layers were pooled and the volume was made up to 50 ml with simulated saliva solution and analyzed spectrophotometrically for insulin content at 276 nm [22]. To check the structural integrity of the insulin after the entrapment procedure, a polyacrylamide gel electrophoresis (PAGE) analysis of native insulin and insulin released from nanoparticles was performed.

3.3.3. Determination of bioadhesion

The polymeric nanoparticles were evaluated for their bioadhesion by the *in situ* method described by Ranga Rao and Buri [23]. Fifty milligrams of nanoparticles were spread uniformly on the mucosa of a 6-cm long piece of rabbit intestine. The intestine was placed in a dessicator maintained at >80% relative humidity at room temperature (28 ± 2 °C) for 20 minutes to

allow for the hydration of polymeric nanoparticles. Mucosal lumen was washed thoroughly with distilled water. The washings were collected, centrifuged and estimated. The ratio of applied and adhered nanoparticles was computed as per cent adhesion.

3.3.4. Drug release profile

The pellet was fixed on to a glass plate with the aid of a silicone adhesive. The glass plate was welded to a glass rod. This assembly was arranged in a manner to keep the pellet in contact with simulated saliva solution contained in a 250 ml glass beaker. Simulated saliva solution was stirred at 50 rpm and maintained at a temperature of 37 ± 1 °C. At periodic intervals, samples were withdrawn and analyzed spectrophotometrically. An equal volume of simulated saliva solution was replaced to make up the volume.

3.3.5. In vivo absorption studies

In vivo studies were performed with P2 and P3 formulations. The selected systems were evaluated for their *in vivo* performance on rats (200–300 g). Diabetes was induced by an intravenous injection of 65 mg/kg streptozotocin in citrate buffer (pH 4.5). A continuous high blood glucose level (300 ± 10 mg/dl) after one-week treatment indicated diabetic condition. Animals were divided into three groups with eight animals in each group. Rats were fasted overnight before experiments and had access to water *ad libitum*. The hypoglycemic response to insulin was measured with an Ames glucometer. One group was kept as control. To the other plain insulin solution (1.4 I.U./kg body weight) was administered intramuscularly. Prior to the administration of pellets to the buccal cavity, rats from the third group were anesthetized by administration of pentobarbitone (30 mg/kg). Blood samples were collected every hour for a total time period of 7 h. Blood samples were centrifuged at 3000 rpm for 3 min to separate serum and glucose concentration was measured. The measured blood glucose concentration was expressed as per cent of the initial concentration. The percent of change in blood glucose expressed was calculated as the initial concentration subtracted from 100. The results are presented in Fig. 3.

Acknowledgement: One of the authors (PV) is thankful to CSIR (New Delhi) for Senior Research Fellowship. The authors would like to acknowledge the Head of the Department of Pharmaceutical Sciences, Doctor Harisingh Gour Vishwavidyalaya, Sagar for providing the necessary facilities to carry out this work.

References

1 Ishida, M.; Machida, Y.; Nambu, N.; Nagai, T.: Chem. Pharm. Bull. **29**, 810 (1981)
2 Nagai, T.; Konishi, R.: J. Control. Rel. **6**, 353 (1987)
3 Nagai. T.; Machida, Y.: Pharm. Int. **6**, 196 (1985)
4 Nagai, T.: Med. Res. Rev. **6**, 227 (1986)
5 Nagai, T.: J. Control. Rel. **2**, 121 (1985)
6 Earle, M. P.: Isr. J. Med. Sci. **8**, 899 (1972)
7 Aungst, B. J.; Rogers, N. J.: Pharm. Res. **5**, 305 (1988)
8 Oh, C. K.; Ritschel, W. A.: Pharm. Res. **5** (Suppl.), 100 (1988)
9 Anders, R.; Merkle, H. P.; Schurr, W.; Ziegler, R.: J. Pharm. Sci. **72**, 1481 (1983)
10 Schurr, W.; Knoll, B.; Ziegler, R.; Anders, R.; Merkle, H. P.: J. Endocrin. Invest. **8**, 41 (1985)
11 Merkle, H. P.; Anders, R.; Sandow, J.; Schurr, W.: Proc. Int. Symp. Controlled Rel. Bioact. Mater. **12**, 85 (1985)
12 Merkle, H. P.; Anders, R.; Sandow, J.; Schurr, W.; in: Davis, S. S.; Illum, L.; Tomlinson, E. (Eds.): Delivery systems for peptide drugs, Nato ASI Series, Series A, Life Science Vol 125, Plenum Press, New York and London, 1986.
13 Wespi, H. J.; Rehsteiner, H. P.: Gynaecologia **162**, 414 (1966)
14 Sjostedt, S.: Acta Obstet. Gynae. Scand. **48** (suppl. 7), 3 (1969)
15 Bergsjo, P.; Jenssen, H.: J. Obstet. Gynaec. Brit. Cwlth. **76**, 131 (1969)
16 Laczi, F.; Mezei, G.; Julesz, J.; Laszlo, F. A.: Int. J. Clin. Pharmacol. Ther. Tox. **18**, 63 (1980)
17 Gu, J.-M.; Robinson, J. R.; Leung, S.-H. S.: Crit. Rev. Therap. Drug Car. Sys. **5**, 21 (1988)
18 Gurny, R.; Meyer, J. M.; Peppas, N. A.: Biomaterials 5, 336 (1984)
19 Peppas, N. A.; Buri, P. A.: J. Control. Rel. **2**, 257 (1985)
20 Junginger, H. E.: Acta Pharm. Technol. **36**, 110 (1990)
21 Duchene, D.; Touchard, F.; Peppas, N. A.: Drug Dev. Ind. Pharm. **14**, 283 (1988)
22 Kim, S. W., Jeong, S. Y., Einink. J. J. D.; Feijen, J., J. Control. Rel. **1**, 56 (1984)
23 Ranga Rao, K. V.; Buri, P.: Int. J. Pharm. **52**, 265 (1989)

Received April 19, 2000
Accepted July 26, 2000

Prof. S. P. Vyas
Pharmaceutics Research Laboratory
Department of Pharmaceutical Sciences
Doctor Harisingh Gour Vishwavidyalaya
Sagar 470 003
India
spvyas@bom6.vsnl.net.in