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Oral Insulin Delivery with Various Grades of HPMC on Non-Diabetic Rats

[1]Jasbir Singh, [1]Gajendra Singh, [3]Nirpinder Singh and [2]Harmeet Kaur

[1]Department of Pharmacy, Pt. B.D. Sharma University of Health Sciences, Rohtak-124001, Haryana, India

[2]Department of Pharmacy, P.D.M. College of Pharmacy, Srai Aurangabad, Bahadurgarh-124507, Haryana, India

[3]Faculty of Applied Sciences, Guru Nanak Dev University, Amritsar-143005, Punjab, India

Corresponding Author: Jasbir Singh, Department of Pharmacy, Pt. B.D. Sharma University of Health Sciences, Rohtak-124001, Haryana, India

ABSTRACT

The aim of the research was to study the potential of various grades of hydroxypropylmethyl cellulose (HPMC) in enhancing oral bioavailability of insulin. Enteric coated HPMC-insulin granules (HPMC-insulin granules), enteric coated control granules (control granules or placebo) of only HPMC and control (peroral) solution of zinc insulin in glycerine IP were tested *in vivo* in rats. An assay value of 99.6±1.6% of HPMC-insulin granules confirmed the minimal insulin degradation during granulation/coating process. *In vivo* studies showed maximum blood glucose lowering with K100LV-insulin granules corresponding to a relative pharmacological efficacy of 1.399±0.138% and absolute pharmacological efficacy of 0.537±0.059%. In contrast, neither control granules nor control (peroral) solution showed a comparable effect. The multiple comparison post-hoc test, LSD (p-level of 0.05), showed a significant difference of K100LV, E50LV, E5LV, E4M-insulin granules from control (untreated) and of only K100LV, E50LV-insulin granules from control (peroral) solution on grades vs. controls basis. On grades vs. grades basis, the potential of HPMCs in lowering of blood glucose levels was finalized as K100LV>E50LV>E5LV≅E4M> K4M>K100M. It is concluded that low viscosity grades of HPMC are efficient in enhancing oral insulin absorption as compared to very low and high viscosity grades. However, viscosity appears to mask effect of substitution ratio on insulin absorption.

Key words: Viscosity, substitution ratio, pharmacological efficacy, blood glucose level, absorption

INTRODUCTION

In recent years, a spurt of research and development has been conducted involving a wide range of protein therapeutics. Although, the administration of proteins by injection is the most effective mean of delivery *in vivo* but patient tolerance of multiple injections is very poor. In addition, the administration of drugs *via* injection routes is a skilled job and requires training; this skill and training may not always be transferable to patients. In cases where protein drugs have a life-saving role, the administration by the injection route is highly accepted. However, in cases where protein drugs are just one of several possible therapies for long use, injections of proteins and peptides are unlikely to be accepted by the patients. Therefore, alternative routes of protein and peptide delivery need to be explored and developed. Among proteins, oral delivery of insulin has

received the widest attention, yet no currently available oral insulin preparation exists. Other alternatives may include the buccal, nasal, oral, pulmonary, rectal and ocular routes. Without exception, these routes are less effective than the parenteral routes of administration. However, these are still far more attractive than the parenteral routes because they offer convenience to patients. Out of these all, oral route is particularly attractive because it is the most convenient, quick and is self-contained. Watts and co-workers proposed drug delivery system for colonic delivery of insulin which releases insulin from proximal colon. The improved bioavailability of insulin results from a combined effect of absorption promoters, dispersing agents and by-pass of liver (Watts and Illum, 2001).

In normal physiology, the 100% insulin that is secreted by the pancreas enters portal circulation where liver uses an estimated 50-80% and rest enters systemic circulation from the liver (Agarwal and Khan, 2001). Even colon targeted or buccal-spray insulin do not reach liver like natural pancreatic insulin. The ratio of plasma insulin in portal circulation versus that in peripheral circulation may vary from 2- to 3-fold (Porksen *et al*., 2002; Arbit and Kidron, 2009). The physiological hypoglycaemic effect of insulin is due to suppression of hepatic glucose production that is enhanced by the increase in glucose use caused by lower insulin levels in peripheral circulation (Satake *et al*., 2002; Camacho *et al*., 2004). So if we make oral insulin to pass through liver, the real physiological effect can be produced which is not possible with colon targeted insulin.

The need to increase absorption rate of insulin across brush-border mucosa appears less as it is absorbed across mucosa normally by passive transport (transcellular endocytosis) (Ziv and Bendayan, 2000). No doubt various polymers have increased insulin bioavailability significantly by paracellular pathway (Tuesca *et al*., 2008). Increasing absorption by permeation enhancer through paracellular way have also resulted into damage to integrity of cells as normal insulin insertion into the lumen of rat duodenum and colon presented evidences of stress or slight degeneration of cellular membrane (Cano-Cebrian *et al*., 2005). The need is to protect insulin from luminal enzymes as it is known that upon ingestion, insulin is subjected to acid-catalyzed degradation in the stomach, luminal degradation in the intestine and intracellular (cytosolic) degradation but not to enzymatic degradation by brush-border enzymes (Agarwal and Khan, 2001).

The application of mucoadhesive polymer has been proposed in present study for making intimate contact with small intestine brush border layer that will facilitate absorption as well as limit exposure of insulin to luminal contents. Unlike colonic insulin delivery, the absorption of insulin from small intestine will also prevent hyperinsulinemia condition which is being foreseen as a problem in all other routes proposed for insulin delivery (Hsu *et al*., 2007). In last few years, pectin, carbopols, polyvinyl alcohol (PVA), chitosan and its derivatives (with thiol groups) and various other mucoadhesive polymers have been checked for their potential in increasing oral bioavailability of insulin. Out of these, chitosan derivatives like thiolated chitosan, poly(MAA-g-EG) hydrogels have shown tremendous success in enhancing absorption (Hosny *et al*., 2002; Bernkop-Schnürch, 2000, 2004; Krauland *et al*., 2004; Mahkam, 2010). But chitosan and its derivatives have not been still recognised as safe ingredients under GRAS (generally recognised as safe) notifications. Furthermore, chitosan and its derivatives show pH dependent mucoadhesive behaviour. In present study, we used GRAS notified polymer i.e., HPMC which is known for its pH independent mucoadhesive properties. HPMC matrix tablets have shown slow and steady erosion type release of soluble drug molecules in wide pH range (1.2 to 7.5) and most often macromolecules do also show similar type of release from swellable matrices (Singh *et al*., 2011).

HPMC is available in various viscosity grades ranging from very low viscosity to very high viscosity; so, dependence of insulin absorption on viscosity has been stressed in this work. Simultaneously, effect of substitution ratio has also been tried to correlate with insulin absorption. The use of permeation enhancers and/or enzyme inhibitors (Yamamoto *et al.*, 1994) was avoided due to several side effects associated with their own absorption and chances of systemic intoxication (Krauland *et al.*, 2004). As HPMCs provided by Colorcon Asia Pvt. Ltd, India were for non-clinical purpose only, so search was restricted to animal studies.

MATERIALS AND METHODS

Materials: Zinc insulin (particle size <1 μm, 1 IU = 0.035 mg, Novo Nordisk, India) ready for i.v. Injection purpose was used in present study. HPMCs (Methocel™, Colorcon Asia Pvt. Ltd., India) and Eudragit™ L100 and L100-55 (Röhm Pharma, Germany) were used as matrix polymer and coating polymers for granules preparation. The required phosphate buffers were prepared as per instructions of Indian Pharmacopoeia 1996 (IP'96) using analytical grade chamicals from SD Fine chemicals, India and LobaChemie, India.

Sugarchek™ glucometer (Wockhardt Ltd.), Hitachi U2800 spectrophotometer, LabIndia dissolution rate testing apparatus, Nichipette micro pipettes, Sartorius electronic balance LE324S etc., were basic instruments used at various stages in research. Sieves no. 22 (0.710 mm), 30 (0.500 mm) and 44 (0.355 mm) of BS standard were used for making and sorting granules. Blunt tip SS needles (No. 18; inner dia. 0.838 mm) covered with siliconized oral feeding tube (No.6) of approx. 1.5 inches length were used for rats oral gavage.

The *in vitro* studies were completed at Guru Nanak Dev University, Amritsar before 2006 end while animal studies completed in mid-2009 after taking all clearances form institutional ethics committee at Pt. BDS University of Health Sciences, Rohtak.

Concentrating insulin process: The concentrating process of zinc insulin was carried out to adjust the insulin dose in a smallest feasible amount of HPMC. Concentrated insulin was not stored to avoid any further stability related studies. Various concentrating processes are known for concentrating insulin like high-performance liquid chromatography, ultrafiltration, centrifugation and even evaporation at various stages in industrial processes. The efficiency of ultrafiltration and centrifugation are considered high compared to chromatography. As we had to concentrate purified insulin only, so centrifugation process was adopted. The modified industrial centrifugation process as per Datar and Rosen was carried out using pooled suspensions of zinc insulin and a laboratory centrifuge at 6500x g for 3 h (Datar and Rosen, 1990). Post-centrifugation, the supernatant and concentrate (insulin phase) were analysed for insulin amounts using Singh and Singh analytical method (Singh and Singh, 2009). The concentrating process was run thrice with 100 mL pooled quantity each time to observe overall variation and effect on insulin assay value.

Experimental designs

Selection of HPMC grades: As in Table 1, six grades of HPMC among eleven grades (divided into 4 groups) provided by the Colorcon Company were used in study covering wide viscosity range. Instead of referring viscosity values each time in further sections, all the grades have been mentioned with their corresponding grade names prefixed (E or K) and suffixed (LV or M) appropriately as described by company.

Table 1: Grouping of HPMC grades on viscosity and substitution ratio basis

HPMC grades	Viscosity (Cps)	Group	Methoxyl%+hydroxypropyl#%	Remark
E3-LV	3	GROUP I	29.2+8.4	Low viscosity and medium substitution ratio grade
E5-LV*	5		29.1+7.7	
E6-LV	6		29.1+8.3	
E15-LV	15		29.1+9.4	
E50-LV*	50		29.0+8.5	
K100-LV*	100	GROUP II	22.8+9.6	Low viscosity and low substitution ratio grade
K4M*	4000	GROUP III	22.9+8.3	High viscosity and low substitution ratio grade
K15M	15000		22.8+8.8	
K100M*	100000		23.3+10.8	
E4M*	3000-5600	GROUP IV	28.8+8.7	High viscosity and medium substitution ratio grade
E10M	7500-14000		28.6+8.9	

*Grades used for studies, #Actual reported values by Colorcon Asia Pvt. Ltd, India for batches provided. Limits as per USP/ Ph Eur. :19.0-24.0% methoxyl, 7.0-12.0% hydroxypropyl for low substitution ratio, 28.0-30.0% methoxyl, 7.0-12.0% hydroxypropyl for medium substitution ratio

Table 2: Composition of single oral doses* (for 150 g rat) used for *in vivo* studies

Ingredients	Uncoated HPMC-insulin granules	Uncoated control granules (placebo)	Control (peroral) solution
Zinc Insulin	0.788 mg	--	0.788 mg
HPMC	179.964 mg	179.964 mg	--
Glycerine IP	--	--	0.2 mL

*Enteric coating resulted into additional 10% increase in theoretical weight of granules

Selection of doses for *in vivo* studies: The per dose compositions of enteric coated HPMC-insulin granules (HPMC-insulin granules), enteric coated control granules (control granules or placebo) and the control (peroral) solution for average 150 g rat are listed in Table 2.

Preparation of granules: The sufficient quantities of concentrated zinc insulin and HPMC were mixed in multiples of one oral insulin dose as proposed in Table 2. The mixture was passed through a sieve set of 10-16-22 BS standard. The 16-22 sized granules after vacuum drying in a desiccator at normal lab temperature were compressed to big slugs by single punch tablet-making machine. The slugs were then crushed and sieved through a sieve set of 22-30-44 BS standard. Thereafter, the granular mass ranging between 30-40 sieves was enteric coated with 50:50% composition of Eudragit L100-55 and Eudragit L100 in a 3% w/v acetonic solution and air dried using lab developed fluid bed dryer at air pressure of 1.8±0.2 kg cm^{-2}. Coating was continued until an average theoretical weight increase of ≈10% had been achieved. The purpose of enteric coating was to prevent insulin release in stomach and making effective release above pH 5.5 as L100-55 and L100 start dissolving within a pH range of 5.5-6.0. The enteric-coated granules were again sieved through 30-40 sieve set to get final granules between 0.355-0.500 mm size for oral administration to rats. The procedure was repeated similarly for preparation of other HPMC-insulin granules. The control granules were prepared of only E4M grade of HPMC without addition of insulin concentrate and using demineralized water as granulating agent.

Effect of granulation/coating process: As the claimed amount of zinc insulin in single oral dose for 150 g rat was 0.788 mg (22.444 IU) and granules were checked for this amount after granulation/coating process in phosphate buffer of pH 7.0. The analytical method used was as

standardised by Singh and Singh (2009). The method was repeated in triplicate for HPMC-insulin granules chosen randomly. The Mean±SD alongwith RSD of % assay were reported to explain variance.

Preparation of insulin solutions for intravenous and subcutaneous injection: Intravenous and subcutaneous injections of zinc insulin suspension served as positive controls for absolute and relative pharmacological efficacies determination. For i.v. injection 0.035 mg kg^{-1} b.wt. (1 IU kg^{-1} b.wt.) and for s.c. injection 0.070 mg kg^{-1} b.wt. (2.0 IU kg^{-1} b.wt.) of insulin were dissolved to final makeup of 0.1 mL in sterile 154 mM phosphate buffered saline pH 7.5 previously filtered through G-5 sintered glass filter (1-2 μm, Borosil, India) and subsequently injected.

***In vitro* release studies:** HPMC-insulin granules of four grades; E5LV, K100LV, E4M and K100M were subjected to *in vitro* dissolution studies in triplicate in pH 1.2 and 6.6. The paddle type dissolution rate testing apparatus complying with standards of IP'96 was used. The release was reported as % insulin release (n = 3) from granules (equivalent to 450 IU insulin) in 500 mL of medium up to 8 h.

***In vivo* evaluation of granules:** The animal studies were approved by Institutional Animal Ethics Committee, PGIMS, Rohtak, India and adhered to the guidelines of lab animal care. Non-diabetic, white albino rats of 150±10 g weight were purchased from approved source Hisar Agriculture University, Haryana. Total 11 groups each consisting of 6 rats were used for *in vivo* studies. Six groups received orally HPMC-insulin granules (each made of different HPMC grade) at an insulin dose of 5.252 mg kg^{-1} b.wt. (150 IU kg^{-1} b.wt.), suspended in glycerine IP for easy administration. Four other groups received s.c. injection/dose, i.v. Injection/dose, control (peroral) solution and control granules for relative, absolute pharmacological reference and controls. One group was kept as control (untreated). Blood samples were withdrawn from tail vein immediately prior to administration of the dose as the t = 0 h value and subsequently at 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0 and 24 h post administration of doses. Additional samplings at 0.5 h interval were done for i.v. and s.c. injections/doses to note their early possibility of pharmacological response. The dosed rats were fasted for 12 h and kept in cages with free access to water. Blood glucose levels were immediately determined using the blood glucose reader or glucometer (SugarCheck, Wockhardt, India) (Krauland *et al*., 2004). The initial blood glucose levels were regarded as 100% and all other levels referred to them as % of initial level.

The Area above Curve (AAC) values of plots of blood glucose against time were calculated for each control and HPMC-insulin granules using control granules (placebo) curve as baseline curve (Nakamura *et al*., 2004). The relative pharmacological efficacy of insulin was determined as the ratio of AAC (pharmacological) for oral dose and AAC (pharmacological) for subcutaneous injections multiplied by ratio of s.c. dose and oral dose, whereas absolute pharmacological efficacy was determined as the ratio of AAC (pharmacological) for oral dose and AAC (Pharmacological) for intravenous injections multiplied by ratio of i.v. dose and oral dose (Krauland *et al*., 2004). So, relative pharmacological efficacy calculated referred to the s.c. injection results, whereas the absolute pharmacological efficacy to i.v. injection.

Statistical data analysis: The multiple comparisons post-hoc test, Least Square Difference (LSD) was applied to explain the difference of grades (6 grades as 6 groups) from each other

and from controls (control (untreated) and control (peroral)) (Norman and Streiner, 2008). A p-level of 0.05 was considered as the minimal level of significance. Calculations were done using SPSS 16.0.1.

RESULTS

Concentrating insulin process: As in Table 3, the overall yield (% Assay) for concentrate or insulin phase remained 78.3±0.3 (Mean±SD; n = 3) in our method which is very low compared to 99.5% step yield of industrial process for insulin (Datar and Rosen, 1990). Net 9.5±0.2 (Mean±SD; n = 3), % loss was observed in the process. The RSD for both yield and % loss was found less than 2.0% in an overall process of centrifugation.

Effect of granulation/coating process: The actual theoretical weight increase observed was ranging between 9.8-11.5% on an overall for all the granules. Table 3 shows the insulin % assay values obtained for three randomly chosen samples of HPMC-insulin granules. The effect of granulation/coating process was minimal on insulin composition in granules as clear from 99.6% assay value. The RSD less than 2.0% also indicated good stability of insulin during granulation/coating process.

***In vitro* release studies:** On an overall, no insulin release was observed in pH 1.2 up to 8 h from any of the four test HPMC-insulin granules. In pH 6.6 (Fig. 3), gradual increase in insulin release rate was observed for all the four grades. The insulin release >85% was observed during first 3.0 h for E5LV and K100 LV. In E4M and K100M this release was not observed even up to 4 h. All the grades showed almost complete (>85%) release upto last time point i.e., 8 h.

***In vivo* evaluation of granules:** To confirm the usefulness of HPMC in increasing oral absorption of insulin, HPMC-insulin granules were tested *in vivo* in rats. In comparison, insulin was applied orally to rats in solution. Furthermore, insulin was injected intravenously and subcutaneously. The response as % change in blood glucose level w.r.t. initial level i.e., t = 0 h following the i.v., s.c. injections/doses and controls have been shown in Fig. 1. Similarly, the results as % change in blood glucose level w.r.t. initial level (t = 0 h) of the orally administered HPMC-insulin granules of different HPMC grades have been shown in Fig. 2.

In Fig. 2, HPMC-insulin granules led to different strength of responses with significant to slight decrease in blood glucose levels except K100M where almost no change observed. Among various

Table 3: Analytical data and amount recovered of insulin in centrifugation and granulation/coating processes

Processes	Dilution factor	IU in supernatant phase 85 mL/ (%Assay)	RSD	Dilution factor	IU in Insulin phase 15 mL/ (% Assay)	RSD	% loss (Mean±SD; n = 3)
Centrifugation[@]	20	487.7±5.9/ (12.2±0.1)	1.2/(1.2)	25*25	3132.7±12.1/ (78.3±0.3)	0.4/(0.4)	9.5±0.2
Granulation/ Coating[#]	Dilution factor	IU predicted/ (%Assay)	RSD	--	--	--	--
	10*10	22.352±0.363/ (99.588±1.618)	1.6/(1.6)	--	--	--	--

Each Conc. values is Mean±SD for n = 3 except dilution factor. Equation applied for assay was AUAC = -0.009+71.112xConc. (Laboratory developed standard equation; unpublished). [@]For centrifugation: claimed amount in pooled qty (100 mL) was 4000 IU (140 mg) of zinc insulin, [#]For granulation/ coating: claimed or labelled amount was 22.444 IU (0.788 mg) of zinc insulin

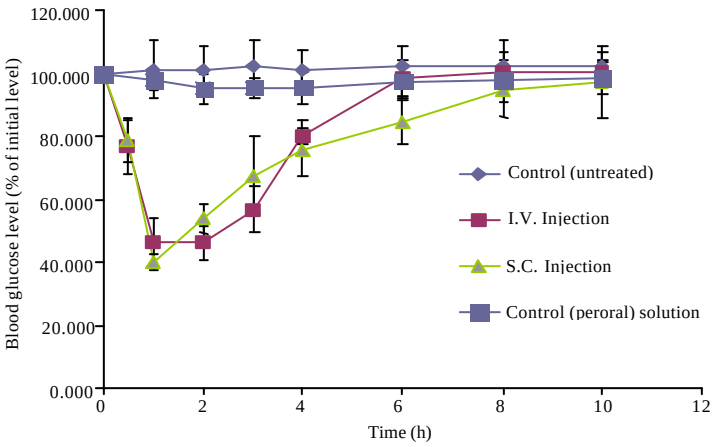

Fig. 1: % Change in blood glucose levels for different controls or references

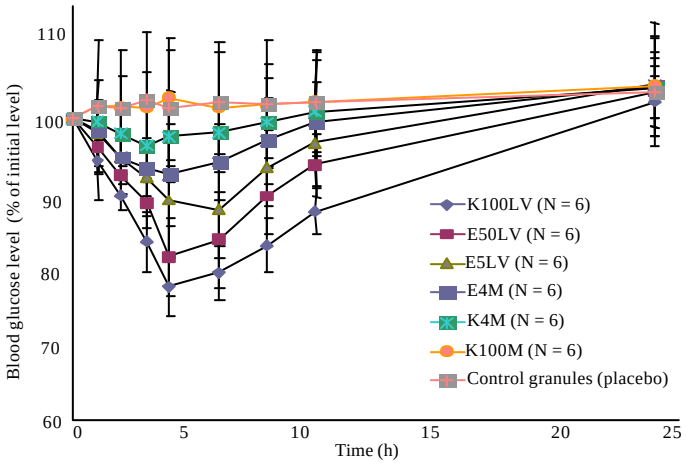

Fig. 2: % Change in blood glucose levels for HPMC-insulin granules of various HPMC grades

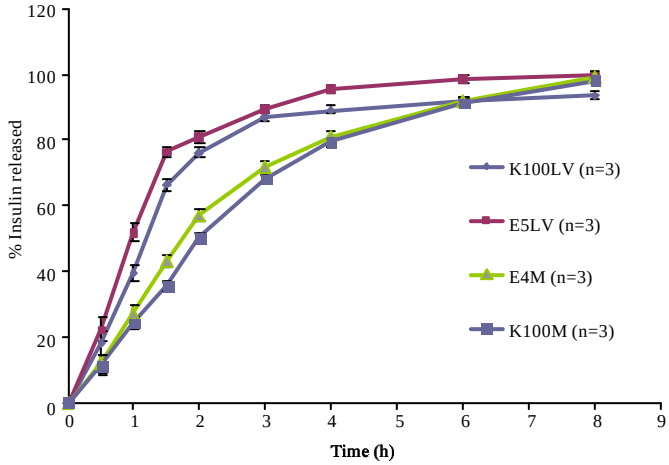

Fig. 3: *In vitro* release of insulin from various HPMC-insulin granules in pH 6.6

Table 4: Main pharmacokinetic parameters after administration of control (peroral) solution, I.V. injection and S.C. Injection

Parameters	Control*(placebo) granules	Control (peroral) solution	I.V. injection	S.C. injection
AAC_{0-24}	--	6.833±4.143	190.167±94.998	230.417±38.205
AAC_{0-10}	--	8.333±5.888	180.000±26.822	200.417±25.516
t_{min}	--	--	2 h	1 h
Relative pharmacological efficacy %	--	0.040±0.024	--	100.000
Absolute pharmacological efficacy %	--	0.029±0.021	100.000	--

Values are Mean±SD (n = 6). *Acted as reference for AAC (Area Above Curve) calculation for all other controls and grades under investigation

Table 5: Main pharmacokinetic parameters after oral administration of enteric coated hpmc-insulin granules of various HPMC grades

Parameters	K100LV	E50LV	E5LV	E4M	K4M	K100M
AAC_{0-24}	241.500±23.763	172.167±22.827	155.000±21.520	94.500±9.268	53.083±12.436	50.417±5.324
AAC_{0-10}	153.167±16.804	120.000±13.035	101.667±11.479	68.000±9.154	37.083±14.800	22.750±12.517
t_{min}	4 h	4 h	6 h	3 h	3 h	3 h
Relative pharmacological efficacy %	1.399±0.138	0.988±0.132	0.898±0.125	0.548±0.054	0.308±0.072	0.292±0.031
Absolute pharmacological efficacy %	0.537±0.059	0.421±0.046	0.357±0.040	0.239±0.032	0.130±0.052	0.080±0.044

Values are Mean±SD (n = 6)

grades, blood glucose lowering effect is maximum for K100LV followed by E50LV and then E5LV. The E4M is showing somewhat intermediary effect while K4M least effect. The maximum pharmacological effect achieved is after 4 h with a decrease of blood glucose level to 76.333±2.582% (Mean±SD; n = 6) in case of K100LV. In all the cases, except K100M, blood glucose levels reached the initial levels upto 24 h of dosage administration but not upto 10 h.

Various pharmacokinetic parameters derived from blood glucose vs. time profiles have been shown for controls (insulin containing) and HPMC-insulin granules in Table 4 and 5. Insulin given as control (peroral) solution did not show any significant effect and the blood glucose levels are in accordance to the normal physiological fluctuations of control (untreated). Pharmacological response after i.v. and s.c. injections had a minimum after 2 h and 1h with 46.116±5.456% and 39.909±2.600% of the initial blood glucose level, respectively. The effect, however, lasted only for 6 and 8 h (Fig. 1).

The maximum and minimum relative pharmacological efficacies are 1.399±0.138% (Mean±SD; n = 6) in case of K100LV and 0.292±0.031% (Mean±SD; n = 6) for K100M as compared to 0.040±0.024% (Mean±SD; n = 6) for control (peroral) solution and 100% for s.c. injection. Similarly, the maximum and minimum absolute pharmacological efficacies are 0.537±0.059% (Mean±SD; n = 6) for K100LV and 0.080±0.044% (Mean±SD; n = 6) for K100M as compared to 0.029±0.021% (Mean±SD; n = 6) for control (peroral) solution and 100% for i.v. injection. The values for both pharmacological efficacies are given in Table 4 and 5 for controls and grades. Thus K100LV increased absorption 35 times on relative basis and 18 times on absolute basis in comparison to control (peroral) solution indicating potential of K100LV in enhancing oral bioavailability of insulin. Depending upon this data the order may be presented primarily as:

K100LV>E50LV>E5LV>E4M>K4M>K100M>control (peroral) solution

The differences in grades and controls analysed statistically with multiple group comparison, LSD test, at p-level of 0.05 using % of initial blood glucose levels as response variables have been

Table 6: Post-Hoc (LSD) test for multiple comparisons between different HPMC grades/controls

Secondary group (J)	Primary group (I)							
	K100LV	E50LV	E5LV	E4M	K4M	K100M	Control[@]	Control[#]
K100LV	--	3.546	6.536*	8.480*	10.695*	13.236*	13.053*	8.792*
		(0.118)	(0.005)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
E50LV	-3.546	--	2.990	4.934*	7.149*	9.690*	9.506*	5.246*
	(0.118)		(0.186)	(0.031)	(0.002)	(0.000)	(0.000)	(0.022)
E5LV	-6.536*	-2.990	--	1.944	4.159	6.700*	6.516*	2.256
	(0.005)	(0.186)		(0.388)	(0.068)	(0.004)	(0.005)	(0.317)
E4M	-8.480*	-4.934*	-1.944	--	2.215	4.756*	4.573*	0.312
	(0.000)	(0.031)	(0.388)		(0.326)	(0.037)	(0.045)	(0.889)
K4M	-10.695*	-7.149*	-4.159	-2.216	--	2.541	2.357	-1.903
	(0.000)	(0.002)	(0.068)	(0.326)		(0.260)	(0.296)	(0.398)
K100M	-13.236*	-9.690*	-6.700*	-4.757*	-2.541	--	-0.184	-4.444
	(0.000)	(0.000)	(0.004)	(0.037)	(0.260)		(0.935)	(0.051)
Control[@]	-13.053*	-9.506*	-6.516*	-4.573*	-2.357	0.183	--	-4.260
	(0.000)	(0.000)	(0.005)	(0.045)	(0.296)	(0.935)		(0.061)
Control[#]	-8.792*	-5.246*	-2.256	-0.312	1.903	4.444	4.260	--
	(0.000)	(0.022)	(0.317)	(0.889)	(0.398)	(0.051)	(0.061)	

*The mean difference is significant at the 0.05 level. Values in parenthesis are significant for each comparison. [@]Control (untreated). [#]Control soln. (peroral)

given in Table 6. The values shown are mean difference values for grades/controls treated as primary and secondary groups one by one. It is apparent from the data that significant difference exists for K100LV, E50LV, E5LV, E4M-insulin granules from control (untreated) and for K100LV, E50LV-insulin granules from control (peroral) solution. This shows that K100LV and E50LV are significantly different from both controls while E5LV and E4M are significantly different from only control (untreated). Hence, K100LV and E50LV increased absorption remarkably while E5LV and E4M moderately. The remaining K4M and K100M enhanced absorption upto so small extent that their responses are not differing significantly even from control (untreated). Thus order on basis of grades vs. controls may be spotted or categorised as:

K100LV, E50LV > E5LV, E4M > K4M, K100M

For ease in understanding, the above order may be pronounced in terms of effect (blood glucose lowering) categories as remarkable effect category, moderate effect category and minor/ or no effect category.

Now after grades vs. controls comparison, the categorised grades may be analysed further for inter-grades comparison. At first the category showing remarkable effect includes, low viscosity, K100LV (Group II, K series) and low viscosity, E50LV (Group I, E series) grades (Table 1). The K100LV is showing significant difference from all grades except E50LV (significance value 0.118) but E50LV is having maximum similarity to E5LV (significant value of 0.186) (Table 6). So, both K100LV and E50LV may be treated as different response groups of remarkable effect category.

The second, moderate effect category also includes the very low viscosity, E5LV (Group I, E series) and high viscosity, E4M (Group IV, E series) grades (Table 1). E5LV is showing strongest similarity for E4M (significance value of 0.388) and similarly E4M for E5LV (significant value

Table 7: Effect of HPMC viscosity and substitution ratio on Oral insulin absorption

Group II (K series)	> Group I (E series)		≥Group IV (E series)	> Group III (K series)	
K100LV	> E50LV	> E5LV	≅ E4M	> K4M	> K100M
100 cps	50 cps	5cps	4300cps	4000 cps	100000 cps
(Low viscosity)	(Low viscosity)	(Very low viscosity)	(High Viscosity)	(High viscosity)	(Very high viscosity)
Low sub.	Med Sub.	Med Sub.		Low Sub.	Low Sub.

of 0.388) (Table 6). Thus responses of E5LV and E4M as grades may be considered equivalent i.e., E5LV≅E4M. This equivalence of individual grades shall not be confused with Group equivalence i.e. Group I≠Group IV, as Group I is representing to large E series, having E5LV as well as E50LV and in no way E4M is equivalent to E50LV. However, such situation may be represented in brief as Group I≥Group IV in producing response, as few grades may show similarity (like E5LV≅E4M) but not all (like E50LV≠E4M in this study).

The minor effect category includes high viscosity, K4M (Group III, K series) and very high viscosity, K100M (Group III, K series) grades (Table 1). The K4M is showing maximum similarity to E4M (significance value 0.326) and K100M to K4M (significance value 0.260) (Table 6), so both are conforming to different response groups. The situation may be represented here as K4M>K100M.

Thus overall order in terms of grades and groups may be represented either as:

$$K100LV > E50LV > E5LV \cong E4M > K4M > K100M \text{ and}$$

$$\text{Group II (K series)} > \text{Group I (E series)} \geq \text{Group IV (E series)} > \text{Group III (K series)}$$

or correlated to viscosity and substitution ratio as in Table 7.

DISCUSSION

The concentrating process was done with a laboratory centrifuge to adjust insulin dose in smallest possible mass of HPMC. The process of concentrating by centrifugation was not as efficient as industrial process described by Datar and Rosen (1990) but adopted being fast and simple. Table 3 is showing the recovered quantity of zinc insulin after centrifugation. The low % yield in our case may be due to use of laboratory centrifuge instead of high-speed industrial centrifuge.

The effect of granulation/coating was minimal as from the data in Table 3 indicating good stability of insulin during process. The normal temperature instead of high temperatures during process may be responsible for low insulin degradation. Further, entrapment of zinc insuhin in solid matrix carrier (HPMC) may be a rational answer for stability of zinc insulin. The fast release of insuhin from E5LV, K100LV compared to E4M, K100M granules may be due to different viscosity categories. The gel layer in former, due to low viscosity, might have washed away easily which was otherwise tough to get eroded in later grades. But on an overall, the results of insulin release were not widely different. These results are consistent to previous findings of various researchers for solid dosage formulations like pellets, tablets having higher concentrations of HPMCs (Campos-Aldrete and Villafuerte-Robles, 1997; Patel and Patel, 2007).

Figure 1, 2 and Table 4, 5 are showing % lowering of blood glucose levels and pharmacological efficacies of administered controls and HPMC-insulin granules. The relative

pharmacological efficacy of 1.399±0.138% from K100LV granules versus 1.69±0.42% from thiolated chitosan-insulin tablets reported by Krauland and co-workers (Krauland *et al.*, 2004) suggests almost equivalent potential of K100LV based insulin granules in lowering blood glucose levels. However, this value is very less compare to ≈8.0% relative pharmacological efficacy from P(MAA-g-EG) hydrogels (Nakamura *et al.*, 2004). The possible reason may be the promotion of insulin absorption through paracellular pathway by strong mucoadhesive complexation hydrogels (Kavimandan and Peppas, 2008).

The insulin solutions administered directly into rat ileal segments have reported relative bioavailability of 0.5±0.1% (Morishita *et al.*, 2004). In our case the relative pharmacological efficacy of 0.04±0.024% indicates heavy degradation of insulin in control (peroral) solution from stomach to small intestine. This also suggests strong protection of insulin in K100LV granules which enhanced insulin absorption by 35 times relatively as compared to control (peroral) solution. Relating different grades response (lowering blood glucose) generates a general consolidated order in following manner:

K100LV>E50LV>E5LV>E4M>K4M>K100M>control (peroral) solution

This order is simple and does not explain similarity or dissimilarity in responses between two or more grades even if present. Further, the effect of viscosity and substitution ratio can only be explained if such similarity or dissimilarity of grades can be predicted. The LSD test provided sufficient analysis for grade vs. grades and grade vs. controls comparisons. The LSD test data was processed first as grades vs. controls and then grades vs. grades. This grades vs. controls comparison resulted into categorization of grades into remarkable, moderate and minor or null effect categories. The categories were represented as:

K100LV, E50LV>E5LV, E4M>K4M, K100M

where, K100LV and E50LV belonged to remarkable effect category; E5LV and E4M to moderate effect category and K4M and K100M to minor effect category.

On grades vs. grades comparison basis the above order was resolved further to another order as:

K100LV>E50LV>E5LV ≅ E4M>K4M>K100M

and group wise as:

Group II (K series)>Group I (E series) ≥ Group IV (E series)>Group III (K series)

The reasons for low absorption from E5LV and E4M-granules may be the easy loss of insulin by simple washouts from these very low viscosity grades and inability of insulin to get released from gel matrix of high and very high viscosity grades (K4M and K100M) granules. This inference is further supported by *in vitro* dissolution studies, which showed fast insulin release from E5LV and delayed release from both E4M and K100M based granules in pH 6.6. So, viscosity in both cases may be responsible for lower pharmacological responses but nothing can be said for substitution ratio effect. This statement is further supported from ranking of K4M and K100M grades (high and

very high viscosity) at last in blood glucose lowering and pharmacological efficacies data (Fig. 2, Table 5). This finding is in relevance to findings of Mesiha and Sidhom studies of increase in insulin absorption by medium viscosity hydroxypropylcellulose grade (Mesiha and Sidhom, 1995). The overall order of HPMC grades along with viscosity and subtitution ratio has been represented finally in Table 7.

The effect of substitution is not understandable and it can be said that viscosity masks the effect of substitution ratio. The effect of substitution ratio at very low and high viscosity ranges need further search.

CONCLUSION

As the low viscosity grades of HPMC featured good absorption enhancing properties. Being GRAS notified and known for pH independent behavior, drug delivery systems based on these will be more acceptable. Further studies with wide range of viscosity and medium to low substitution ratio for same viscosity shall be tried.

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