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Biological activity of insulin in GMO gels and the effect of agitation

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Abstract

Glyceryl monooleate (GMO)–water cubic phase gel was previously shown to protect insulin from agitation induced aggregation. However, it is not known if insulin is biologically active in the gel and what effect agitation has on insulin in the gel. Therefore, the objective was to determine the stability of insulin in cubic phase gel in terms of its biological activity in a suitable animal model such as Sprague-Dawley rats. Effect of agitation on biological activity of insulin in cubic phase GMO gel was determined by subcutaneous injections of the agitated and non-agitated gels to two groups of previously fasted rats and measuring the effect on their blood glucose levels. Two groups of rats administered with agitated insulin solution and normal saline were used as controls. The biological activity of insulin was evaluated by comparing AAC (area above the blood glucose level–time curve, in %-h), C_{max} (maximum % decrease in blood glucose levels) and t_{max} (time required to attain C_{max}, in h) values for the four groups of rats. Since cubic phase gel is highly viscous, therapeutic equivalency of insulin in the lamellar phase gel, which converts in situ into cubic phase gel, was compared to insulin solution with normal saline as the control, using AAC, C_{max} and t_{max} of the blood glucose profile. Insulin was biologically active in both agitated and non-agitated gels; however, upon agitation, insulin in solution totally lost its hypoglycemic activity. Agitation of insulin in the cubic phase gel was seen to have very little deleterious effect on its biological activity. Insulin in the lamellar phase gel was not only biologically active but also therapeutically equivalent to insulin solution based on AAC (327.9 ± 100.8 and 431.7 ± 113.3), C_{max} (57.1 ± 7.0 and 70.2 ± 6.5) and t_{max} (2.8 ± 0.7 and 4.0 ± 1.7) for the lamellar phase gel and insulin solution, respectively (no significant difference, $P > 0.05$). In summary, GMO cubic phase gel protected insulin from agitation induced aggregation, and insulin was biologically active in the gel.

Keywords: Cubic phase; GMO; Insulin; In vivo activity; Proteins; Rats; Stability

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1. Introduction

In a previous study, the liquid crystalline cubic phase gel of glyceryl monooleate (GMO) was shown to protect insulin from agitation induced aggregation using ultraviolet (UV) and circular dichroism (CD) spectroscopy (Sadhale and Shah, 1999). CD spectroscopy showed a total loss of native conformation of insulin upon aggregation in solution; in contrast, agitation of insulin in gels for as long as 2 months at 37°C, had little adverse effect on the native conformation of insulin (Sadhale and Shah, 1999). However, it was not known whether this insulin in the gel was still biologically active. Since aggregation of insulin results in the loss of its biological activity, a demonstration of the ability of insulin in the cubic phase gel to lower blood glucose levels in a suitable animal model could be an unequivocal evidence of stabilization of insulin against aggregation by the cubic phase gel. Therefore, the primary goal of this study was to confirm the stability of insulin in the cubic phase gel in terms of its biological activity which is lowering of blood glucose levels. This goal was achieved with the help of three studies.

The specific aim of the first study was to determine the effect of agitation on the biological activity of insulin in the cubic phase gel in a suitable animal model. Various animal models like the alloxan-diabetic rabbit (Tachibana, 1992), the non-obese diabetic (NOD) mouse (Hanafusa et al., 1994), streptozotocin-induced diabetic Sprague-Dawley (SD) rats (Muranushi et al., 1993) and the normal fasted SD rat (Golomb et al., 1993; Shao et al., 1993; Li and Mitra, 1994, 1996) have been used to study insulin-dependent diabetes and its therapy. In the current study, the normal fasted SD rat was used as the animal model to prevent interference from any physiological modifications that could possibly occur in animals with drug-induced diabetes. Two sets of cubic phase gels containing insulin were prepared, and one set of gels were agitated along with an insulin solution at 100 oscillations/min at 37°C. When all the insulin in the solution was found to have aggregated, agitation was stopped. The non-agitated and the agitated cubic phase gels, and the agitated solution were appropriately diluted and separately administered to three groups of previously fasted rats. A fourth group of rats received normal saline as the control. The effect of the administration of these formulations on the blood glucose levels was determined as described below.

Due to the high viscosity of cubic phase gel, it is difficult to inject, however, GMO also forms lamellar phase gel of low viscosity with 3–20% water which can be injected through a syringe, and it is known to absorb water and body fluids in situ to convert into the cubic phase (Larsson, 1989; Ericsson et al., 1991). The lamellar phase gel has also been shown to protect insulin from agitation induced aggregation using UV and CD spectroscopy (Watson et al., 1998). Thus, the specific aim of the second study was to determine the biological activity of insulin in a lamellar phase gel in vivo. The biological activity of insulin was studied by separately administering insulin in a lamellar phase gel, insulin in solution and normal saline as control to three groups of previously fasted SD rats and monitoring the effect on their blood glucose levels. The potential mechanisms by which GMO cubic phase gel protected insulin from agitation induced aggregation has been discussed in the previous report (Sadhale and Shah, 1999). In this study, the GMO gel was shown to be able to protect and deliver the biologically active insulin in vivo, further confirming the stability enhancing properties of GMO gels. Therefore, these results using insulin as a model peptide demonstrate the potential use of liquid crystalline lamellar and cubic phase gels of GMO as delivery matrix for other proteins and peptides.

2. Materials and methods

GMOrphic 80® (GMO, monoolein) was a gift from the Eastman Chemical Company (Rochester, NY). Humulin® (Eli Lilly and Co., IN), ketamine HCl (Ketalar®, Parke-Davis, NJ), xylazine HCl (Rompun®, Miles Inc., KS) and normal saline (Abbott Labs., IL) were purchased from the MUSC Hospital Pharmacy. Monobasic and dibasic sodium phosphates were procured from Curtin Matheson Scientific, Inc. (Houston, TX). Methylene chloride was purchased from Fisher Scientific (Fair Lawn, NJ).

2.1. Preparation of insulin-containing cubic phase gels and solutions

Insulin containing cubic phase gels (GMO:water; 70:30, w/w) were prepared to study the effect of agitation on the in vivo activity of insulin. Gels containing 30 units of insulin per gram of gel were prepared by slowly stirring an appropriate amount of the Humulin® solution with an accurately weighed quantity of GMO which had been previously melted by warming in a water-bath at 45°C. The resultant gel was then centrifuged to remove air bubbles, if any. The cubic phase was completely formed in less than 24 h, which was confirmed by examination for its isotropic nature and phase transitions on heating using hot-stage microscope under a polarized light. Control solutions for this study were prepared by simply diluting Humulin® (100 U/ml) with normal saline to obtain solutions containing 30 U/ml of insulin.

2.2. Effect of agitation on biological activity of insulin in solution and cubic phase gel

Two sets of cubic phase gels containing 30 U/g of insulin were prepared and one set was set aside immediately after preparation while the other set was agitated at 100 oscillations/min at 37°C along with one set of insulin in normal saline (30 U/ml) as described previously (Sadhale and Shah, 1999). Seven days later, when the insulin in solution was found to have totally aggregated, agitation was stopped and the solution was centrifuged to separate the precipitated aggregates of insulin. The agitated gel was then softened by addition of small amounts of methylene chloride which leaves behind an aqueous solution of insulin as described previously (Sadhale and Shah, 1999). These softened agitated gels and the non-agitated gels and the solution were then diluted with normal saline such that 1 ml of each would deliver insulin at a dose of 10 U/kg to each rat.

Fourteen healthy male SD rats, weighing 250–300 g, were randomly assigned to four groups as follows: (1) control group ($n=4$), which would receive normal saline instead of any insulin formulation; (2) agitated gel group ($n=3$), which would receive the diluted agitated insulin gel; (3) non-agitated gel group ($n=3$), which would receive the diluted non-agitated gel; (4) agitated solution group ($n=4$), which would receive clear agitated solution. All the rats were fasted for 16 h prior to the experiment but allowed access to water ad libitum. The rats were anesthetized by an intramuscular injection of a mixture of 50 mg/kg ketamine and 12 mg/kg xylazine. Blood was then drawn from the tail vein by nicking the end of the tail with a sterile scalpel and applying gentle pressure to the tail. Blood glucose levels were measured using an Accu Chek Easy® Blood Glucose Monitor and test strips (Boehringer Mannheim Corp., Indianapolis, IN). Blood glucose levels at -1 and -0.5 h (i.e. 1 and 0.5 h prior to administration) and 0 h were averaged and the mean value considered to be the baseline glucose level and used to normalize the rest of the values. After determining the baseline glucose level, the rats in each group were then subcutaneously injected with 1 ml of the appropriate formulation at a dose of 10 U/kg body weight as per the group assignment. Blood was drawn at predetermined time intervals after dosing as described before and the glucose levels measured with the glucose monitor and analyzed as described later.

2.3. Preparation of insulin-containing lamellar phase gels and solutions

Lamellar phase gels containing 95% GMO were prepared by slowly stirring in an appropriate amount of the insulin solution into accurately weighed, previously molten GMO. The resultant gel had a mucus-like consistency and was free flowing. The lamellar phase of the gel was confirmed by examination under a polarized light, hot-stage microscope for its anisotropic birefringent nature. The lamellar phase gels for the in vivo activity of insulin were prepared such that they could deliver an insulin dose of 10 U/kg body weight by administering 1 ml of the gel.

Insulin solutions used in the in vivo activity study were prepared by simply diluting the Humulin® solution with normal saline such that 1 ml of the solution would deliver insulin at a dose of 10 U/kg body weight.

2.4. In vitro release of insulin from the lamellar phase gel

The lamellar phase gel (5 U/g) was drawn into a 10-ml syringe and fixed amounts were injected into three vials, each containing 10 ml of the release medium (0.1 M phosphate-buffered saline (PBS), pH 7.4). The vials were placed and gently shaken in a water-bath shaker at 37°C, and at predetermined time intervals all the release medium from each vial was sampled and the sample was then analyzed for insulin by high-performance liquid chromatography (HPLC).

2.5. HPLC assay of insulin

Insulin was analyzed by reverse phase HPLC on a Hypersil™ C4 column (5 μm particle size; 3.9 mm i.d. × 300 mm) and eluted with the mobile phase consisting of a mixture of 70% aqueous solution of 0.02 M trichloroacetic acid and 30% acetonitrile, at a flow rate of 1 ml/min. Insulin was detected at 254 nm with a UV detector and linear calibration curves were constructed for 0.625–10 μg/ml of insulin.

2.6. Biological activity of insulin in the lamellar phase gel

Eighteen healthy male SD rats, weighing 250–300 g, were randomly assigned to three groups based on the treatment they would receive. The three groups were: (1) control group ($n = 6$), in which the rats would receive normal saline instead of any insulin formulation; (2) gel group ($n = 6$), in which the rats would receive insulin (10 U/kg body weight) in a lamellar phase gel; and (3) solution group ($n = 6$), in which the rats would receive insulin (10 U/kg body weight) in solution. All the rats were fasted for 16 h prior to the experiment but allowed access to water ad libitum. The rats were anesthetized by an intramuscular injection of a mixture of 50 mg/kg ketamine and 12 mg/kg xylazine. After determining the baseline glucose level as in the previous study, the rats in the gel and solution groups were subcutaneously injected with 1 ml of the appropriate insulin formulation at a dose of 10 U/kg body weight, while those in the control group received a subcutaneous injection of 1 ml of normal saline. Blood was drawn at predetermined time intervals as described before and the glucose levels measured with the glucose monitor.

3. Data analysis: blood glucose level–time profiles

Blood glucose levels were normalized with respect to the baseline blood glucose level which was determined as explained above, and the normalized values were then plotted against time to obtain blood glucose level–time profiles. The efficacy of each formulation was evaluated in terms of area above the blood glucose level–time curve for 9 h (AAC_{0-9}) (Shao et al., 1993; Li and Mitra, 1994; Shao et al., 1994), the maximum percent decrease in blood glucose levels (C_{max}) and time (t_{max}) required to attain C_{max}. Since the drop in blood glucose levels is proportional to blood insulin levels, AUC for insulin level–time curve is proportional to the AAC for the blood glucose level–time curve (Li and Mitra, 1994). Thus AAC is an estimate of the efficacy of the formulation based on a pharmacokinetic–pharmacodynamic correlation. The AAC values were calculated using the linear trapezoidal rule (Touitou and Rubinstein, 1986). C_{max} was calculated as 100 minus the lowest % blood glucose level. The AAC, C_{max} and t_{max} values so obtained are presented as mean ± S.D. and were evaluated for differences by ANOVA at $P < 0.05$.

4. Results

4.1. Effect of agitation on biological activity of insulin in solution and cubic phase gel

Fig. 1 shows the change in blood glucose levels in rats that received the non-agitated insulin gel or the agitated gel, as compared to that observed in rats which received the agitated insulin solution or just normal saline. Rats which received normal saline, but no insulin, did not show a significant change in blood glucose levels. However, rats which received the non-agitated insulin gel dis-

played a rapid drop in blood glucose levels after administration, reaching the nadir ($26.60 \pm 8.42\%$) in 1.33 ± 0.58 h ($n = 3$). Rats which received the agitated insulin gel also displayed a significant drop in blood glucose levels; however, this drop in blood glucose levels was slightly slower than in the rats which received the non-agitated gel. The lowest glucose level ($47.17 \pm 8.13\%$) was reached in 2.67 ± 0.58 h ($n = 3$). In contrast, rats which received the agitated insulin solution did not display any drop in blood glucose levels. In fact, glucose levels in these rats were slightly higher than those observed in control rats that received normal saline. Therefore, insulin in the agitated gel was still biologically active while insulin in solution which was agitated had lost it activity.

As explained before, the equivalency of each formulation was evaluated in terms of AAC for 9 h, C_{max} and t_{max}. Table 1 lists the values for AAC_{0-9}, C_{max} and t_{max} obtained for the rats in this study. The values of all the three parameters are significantly lower in rats that received the agitated gel as compared to those that received the non-agitated gel. Thus it can be inferred that the insulin in the cubic phase gel was still active and that the agitation had very little deleterious effect on insulin. Interestingly, there was no significant difference between AAC_{0-9}, C_{max} and t_{max} for rats that received insulin in the lamellar phase gel in the next study (Table 2) and rats that received the agitated insulin cubic phase gel (Table 1). It is also evident that agitation of insulin in solution led to the total loss of its biological activity due to aggregation.

4.2. In vitro release of insulin from lamellar phase gel

Fig. 2 shows release profile ($n = 3$) of insulin from lamellar phase gel that undergoes transition to cubic phase gel. Insulin was released rather rapidly in the beginning with $63.45 \pm 2.46\%$ being released in 12 h. This burst release could be attributed to the time it takes for the lamellar phase to absorb some of the release medium and convert into the cubic phase. The complete conversion to the cubic phase was confirmed by observing a small sample of the gel under polarized light on hot-stage microscope for its isotropic

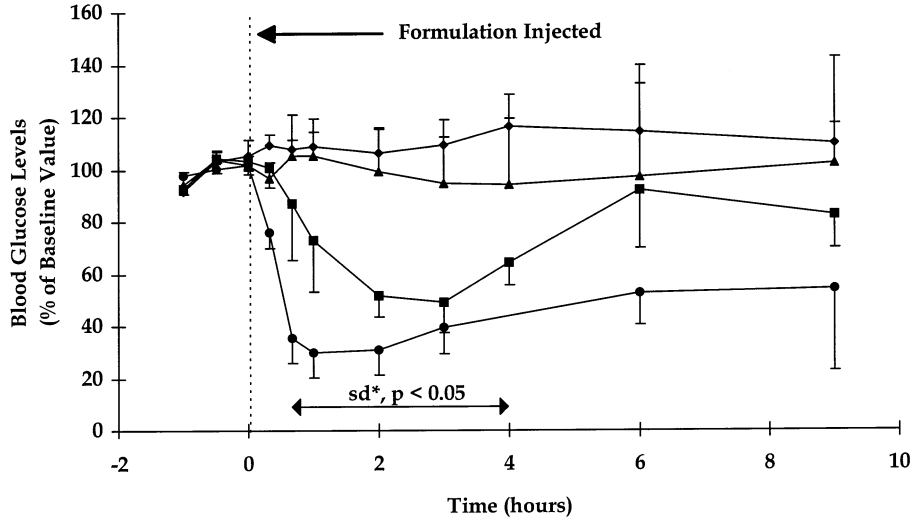

Fig. 1. Effect of agitation on the biological activity of insulin in rats given a subcutaneous injection of (a) the non-agitated insulin cubic phase gel (●) ($n = 3$), (b) the agitated insulin cubic phase gel (■) ($n = 3$), (c) agitated insulin solution (◆) ($n = 4$) and (d) normal saline as control (▲) ($n = 3$). Blood glucose levels for rats given the non-agitated gel and the agitated gel are significantly lower than those in rats given the agitated solution or just normal saline from 40 min to 4 h (denoted by the asterisk on the graph), $P < 0.05$.

Table 1
Comparison of AAC, C_{max} and t_{max} between the rats administered the agitated cubic phase insulin gel to those given non-agitated cubic phase insulin gel ($n = 3$)

Rat	AAC[a] (%-h)		C_{max}[b] (%)		t_{max}[c] (h)	
	Agitated gel	Non-agitated gel	Agitated gel	Non-agitated gel	Agitated gel	Non-agitated gel
1	222.59	401.89	55.3	64.4	3	1
2	296.43	518.94	59.4	74.7	3	2
3	153.57	579.13	43.7	81.1	2	1
Average	224.20[d]	499.99[d]	52.8[d]	73.4[d]	2.7[d]	1.3[d]
S.D.	71.44	90.12	8.1	8.4	0.6	0.6

[a] AAC, area above the blood glucose level–time curve. Values were calculated using the linear trapezoidal rule.
[b] C_{max}, maximum % decrease in blood glucose levels. $C_{max} = 100 - (\text{lowest \% blood glucose level})$.
[c] t_{max}, the time required to attain C_{max}.
[d] Statistically significant difference between the corresponding values for the rats given agitated gel and those given non-agitated gel, $P < 0.05$.

nature. After the initial burst, however, the release slowed down with 100% insulin released in 4 days. From these results it appears that the cubic phase gel does indeed act as a sustained-release delivery system for insulin in vitro, after it is formed from the lamellar phase. The gentle agitation during the release study did not result in aggregation of insulin since the release medium was clear and almost 80% of insulin was released in 24 h.

4.3. Biological activity of insulin in lamellar phase gel

Fig. 3 shows the drop in blood glucose levels after subcutaneous administration of insulin in the lamellar phase gel and in solution as compared with that observed after administration of normal saline as control. There was no significant drop in blood glucose levels in rats that just received normal saline instead of any insulin for-

Table 2
Comparison of AAC, C_{max} and t_{max} between the rats in the lamellar phase gel and the solution groups ($n = 6$)

Rat	AAC[a] (%-h)		C_{max}[b] (%)		t_{max}[c] (h)	
	Gel group	Solution group	Gel group	Solution group	Gel group	Solution group
1	177.47	380.80	54.5	73.4	2	2
2	449.42	311.73	62.7	64.0	2	3
3	406.16	503.73	62.4	68.7	3	3
4	361.27	305.93	47.0	61.6	3	4
5	249.51	521.54	52.0	77.5	4	6
6	323.74	566.17	64.2	76.1	3	6
Average	327.93[d]	431.65[d]	57.1[d]	70.2[d]	2.8[d]	4[d]
S.D.	100.79	113.26	7.0	6.5	0.7	1.7

[a] AAC, area above the blood glucose level–time curve. Values were calculated using the linear trapezoidal rule.
[b] C_{max}, maximum % decrease in blood glucose levels. $C_{max} = 100 - (\text{lowest \% blood glucose level})$.
[c] t_{max}, the time required to attain C_{max}.
[d] No statistically significant difference between the corresponding values for the gel group and the solution group, $P > 0.05$.

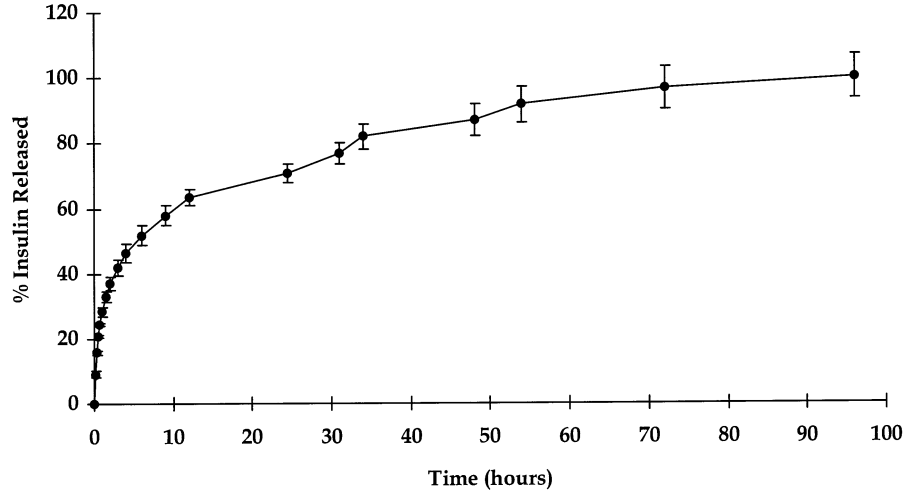

Fig. 2. Release profile of insulin from lamellar phase gel that undergoes transition to cubic phase gel in the release medium ($n = 3$). The release was monitored by injecting the lamellar phase into a vial containing the release medium where it absorbed the medium and converted into the cubic phase. The contents of the vials were agitated at 50 oscillations/min at 37°C.

mulation. The glucose levels stayed at, or around, 100% throughout the duration of the study. However, as expected, in the rats that received insulin solution, the glucose levels started falling rapidly after injection and reached the nadir ($29.78 \pm 6.52\%$) in 4.00 ± 1.67 h ($n = 6$). Rats that received insulin in the lamellar phase gel also showed a similar fall in blood glucose levels. The drop in glucose levels, however, was slower in the beginning and the glucose levels reached their lowest value ($42.88 \pm 7.01\%$) in 2.83 ± 0.75 h ($n = 6$). In both cases, glucose levels went back to their basal level in about 9 h.

The equivalency of each formulation was evaluated in terms of AAC for 9 h, C_{max} and t_{max}, listed

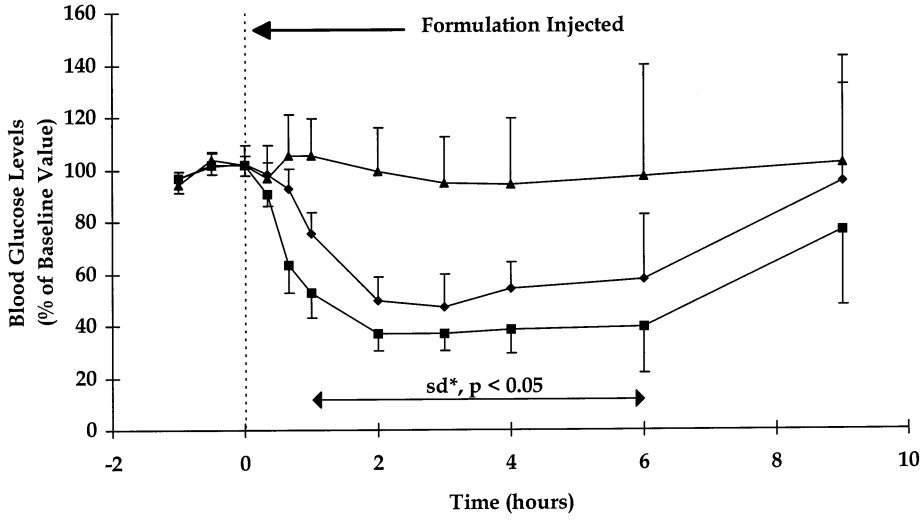

Fig. 3. Blood glucose levels (% of baseline value) in rats from the solution group (■) ($n = 6$), the lamellar phase gel group (◆) ($n = 6$) and the control group (▲) ($n = 6$). Blood glucose levels in rats from the solution group and the gel group from 1 to 6 h are significantly lower than those in the control group (denoted by the asterisk on the graph), $P < 0.05$.

in Table 2. A comparison of the C_{max} and t_{max} values suggests that insulin is released slightly slowly from the gel as compared to solution as expected. However, there is no statistically significant difference between the mean values of AAC_{0-9}, C_{max} and t_{max} for the rats in the gel group and those in the solution group. Thus not only is insulin stable in the lamellar phase gel but it is also biologically active and equivalent in potency to insulin solution. Therefore, the gel and the solution formulations are therapeutically equivalent.

5. Discussion

Aggregation and precipitation of insulin in infusion bags and other delivery devices not only results in obstruction of delivery routes but also, more importantly, in the loss of its biological activity which is lowering of blood glucose levels (Bringer et al., 1981; James et al., 1981; Brange and Havelund, 1983; Lougheed et al., 1983; Brennan et al., 1985; Brange and Langkjaer, 1993). Aggregation of insulin is typical of protein drugs and was previously shown to be prevented by incorporating it in GMO cubic phase gel (Sadhale and Shah, 1999). The gel was not only successful in protecting insulin from agitation-induced aggregation but the secondary structure of insulin in the gel was also found to be intact as confirmed by CD spectroscopy. However, it was not known whether this insulin was still biologically active. Thus, the ability of insulin in the non-agitated as well as agitated cubic phase gel to lower blood glucose levels in vivo found in this study is strong evidence for the stabilization of insulin in the gel against agitation. Administration of both the non-agitated and the agitated insulin gel resulted in a lowering of blood glucose levels in rats; however, administration of the agitated insulin solution had no effect on the blood glucose levels. These results show that agitation had resulted in a total loss of the activity of insulin in solution while insulin in the cubic phase gel seemed to be relatively unaffected by it. There were differences in AAC, C_{max} and t_{max}, for the rats in the non-agitated insulin gel group and the agitated insulin gel group which could have been possibly due to some loss of activity in agitated gels. However, these parameters for agitated cubic phase gel were not significantly different from the parameters for rats in the next study that received insulin in the lamellar phase gel or solution, indicating that agitation of insulin in the cubic phase gel had little deleterious effect on its biological activity.

Although anesthesia is reported to result in stress and release of hormones which may have an impact on glucose homeostasis, in both studies there was no change in blood glucose levels from the baseline levels in rats after administration of normal saline. Also, there was very little variation in baseline glucose values of all the rats during and after anesthesia suggesting that anesthesia had little direct effect on glucose levels, and drop in glucose levels were due to the biological effects of administered insulin in solution or the gel.

Since the extremely viscous nature of the cubic phase gel makes it difficult to inject directly, the less viscous lamellar phase gel which is known to absorb water and convert into the cubic phase in situ was evaluted as a delivery matrix for insulin in terms of in vitro release and in vivo activity of insulin. Fig. 3 shows that administration of insulin in the lamellar phase gel results in a lowering of blood glucose levels similar to that observed upon administration of insulin in solution, and there was no statistically significant difference between blood glucose levels for rats in the solution group and the gel group from 1 to 6 h. The lack of a significant difference between the AAC_{0-9}, C_{max} and t_{max} values for the rats in the solution group and the gel group show that solution and lamellar phase gel formulations of insulin were therapeutically equivalent. However, AAC_{0-9} and C_{max} were slightly smaller for the lamellar phase gel, which however can not be due to lower stability of insulin in it since protection of insulin from agitation induced aggregation in lamellar phase gel has already been demonstrated (Watson et al., 1998). In fact the slower release of insulin in the gel as compared to the immediate systemic availability of insulin from solution may be responsible for this difference, although statistically insignificant.

Insulin was shown to be biologically active in the lamellar phase gel; however, there was a

marked difference between the in vitro release of insulin and the duration of its in vivo activity. While it took 4 days for insulin to be completely (100%) released in vitro, the blood glucose levels returned to their basal level in 9 h, indicating that in vivo insulin may be completely released in less than 9 h. One of the plausible explanation is that, since GMO is susceptible to metabolism by esterases present in various tissues in the body, lamellar phase gel could have been rapidly degraded in the subcutaneous tissue in vivo leading to the rapid release of insulin. Another reason for the rapid release of insulin in vivo could be the slow transition of the lamellar phase to the cubic phase of the gel which actually has the desired sustained-release characteristics. Since the subcutaneous tissue has significantly lower amounts of free water or other fluids compared to the in vitro environment, it may have taken longer for the lamellar phase gel to undergo the transition to the cubic phase. This could result in a faster diffusion and thus rapid release of insulin from the gel in vivo relative to its rate of diffusion and release in vitro. The lack of correlation between the in vitro release and the duration of in vivo activity of insulin indicates that the present in vitro release method for the lamellar phase gel might not be accurate in predicting its in vivo behavior.

Although in this study biological activity of insulin in GMO gels was evaluated as a function of its physical stability, the overall objective was not to develop a sustained-release delivery system for insulin; such a delivery system for insulin is not desirable because it can result in insulin shock due to prolonged hypoglycemia. However, our results demonstrated, using insulin as a model peptide, the stability enhancing properties of liquid crystalline gels of GMO and their potential use as a delivery matrix for other proteins and peptides (Sadhale and Shah, 1999). The potential mechanisms by which GMO cubic phase gel protected insulin from agitation induced aggregation has been discussed in the previous report (Sadhale and Shah, 1999). In this study, the GMO gel was shown to be able to protect and deliver the biologically active insulin in vivo. The cubic phase gel has also been shown to protect peptides from enzymatic degradation in simulated intestinal fluids (Ericsson et al., 1991). The sustained delivery of oligopepetides such as desmopressin and somatostatin from GMO gel after subcutaneous administration has been demonstrated (Ericsson et al., 1991). So far there are no reports of insulin delivery from GMO gels; however, from the results of our study, it appears that GMO gels could potentially be useful as a matrix for oral and parenteral delivery of peptides like insulin. Furthermore, GMO has been approved by the FDA for human consumption and its presence in various products for oral consumption should greatly facilitate its development as a matrix for oral formulations. However, more studies focussing on the mechanism of stabilization of proteins in general, and insulin in particular, in the cubic phase are required.

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