EVALUATING THE EFFECT OF DIFFERENT PLGA MICROSPHERE PREPARATION METHODS ON PHARMACOKINETICS OF EXENATIDE

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Abstract:
Solvent extraction and Co-solvent method have been widely used methods for preparation of Poly (DL-lactic-co-glycolic acid) (PLGA) microspheres. However there were no reports on evaluating the effect of different PLGA microsphere preparation methods on exenatide release and it’s corresponding pharmacokinetics. Four different exenatide PLGA Microspheres ie HSE, USE, SoEX and COS were prepared by following four different approaches. All the four different exenatide PLGA microspheres, solution and Bydureon® was administered through subcutaneous route to male Sprague Dawley rats at different dose. Plasma samples were analysed using LC/MS method. The highest initial burst release was achieved by SoEx MS formulation whereas the transient second burst was observed higher for COS MS formulation. It was observed that that even though the drug release was controlled by polymer degradation, the internal structural changes of microspheres played the most important role than the decrease of polymer Mw. The cumulative release of exenatide from HSE based microspheres was similar to COS MS and higher among the other treatment groups. Upon dose normalization and comparing the peak maximum concentrations (Cmax) achieved by microsphere formulations with Byduron group for COS, HSE, SoEX, USE group was 12.4X, 28.5X, 40.3X and 6X higher whereas the exposure (AUC0-t) achieved by microsphere formulations compared with Byduron group for COS, HSE, SoEX, USE group was 7.3X, 3.4X, 2.8X and 2.8X higher. The detailed PK based evaluation of PLGA based microspheres prepared by different methods in the study provide help in guiding the emulsion-microsphere preparation or other long-effective release systems and also the results of the study reveals another important point that invitro release behaviours of these microspheres were not influenced by different preparation methods but was affected by internal structure evolution. Therefore, COS MS can be evaluated further for developing a once-in-a twoweeks injection of COS MS to replace a BID daily injection of exenatide.

Key Words: Exenatide; PLGA microspheres; Subcutaneous Route; Bydureon

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INTRODUCTION:
Diabetes is one of the common chronic diseases in modern society, therefore the development of antidiabetic drugs and relevant formulations are of primary importance. The central goal in the treatment of type 2 diabetes mellitus (T2DM) is the proper maintenance of glycemic control otherwise there is an increased risk of cardiovascular disease, diabetic nephropathy with micro-albuminuria, microvascular and macrovascular complications (1). Current antidiabetic therapeutic agents cannot be used for sustained long-term glycemic control, additional treatment options for maintaining normoglycemia in T2DM patients are needed (2,3). Therefore in order to satisfy the unmet needs of current anti-diabetic drugs several new classes of anti-diabetics have been approved and one of them is glucagon-like peptide-1 (GLP-1) receptor agonists.

Exenatide (Exendin-4 or EX4), is a 39-amino acid peptide hormone found originally in the salivary secretions of the Gila monster it shares extensive homology and function with mammalian GLP-1, but has a therapeutic advantage in its resistance to degradation by DPP-IV (which breaks down GLP-1 in mammals) therefore allowing for a longer pharmacological half-life and enhanced potency, as a result of which it can be administered by twice-daily subcutaneous (SC) injection (4,5,6). In preclinical studies on comparison with GLP-1, EX-4 has a 20–30-fold longer half-life and 5500-fold greater potency in decreasing plasma glucose (7, 8).

However, the complexity associated with the EX-4 treatment regimen, negatively affects patient compliance which includes the frequency of administration and duration of treatment. To overcome the issue of compliance one strategy that might significantly help is reduction of the required frequency of administration. Two different GLP-1 receptor agonist formulations approved by FDA for diabetes treatment are Byetta® (Exenatide injection) and Bydureon® (Exenatide extended-release microspheres).

Bydureon microsphere, the currently approved once-weekly PLGA microsphere injection was prepared by following water-in-oil (W/O) solvent evaporation method and concerns exist regarding the production of exenatide-derived impurities during the preparation. (9-11). It improved hemoglobin A1c (HbA1c) and reduced fasting plasma glucose, with fewer gastrointestinal side effects. However, a “lag phase” of 7 weeks was observed in the release profiles of Bydureon microspheres which contributes to it’s poor pharmacokinetics and efficacy inspite of the initial burst in the first 2 days due to the loosely bound exenatide on the surface (12-14). In addition, the mean particle size of Bydureon® LAR is about 50 μm, which necessitates the use of painfully large, 23-gauge needles for the SC administration. In addition, the weekly dose of EX-4 in Bydureon® (i.e. a single dose of 2 mg/human) is much higher (14–28-fold) than that of Byetta® (i.e. 5–10 mg/human twice a day, which corresponds to a total weekly dose of 70–140 mg/human).

To improve the delivery of Exentaide from PLGA microspheres and aiming to develop a better formulation than the Bydureon microsphere. Various research groups have worked such as a biweekly dosage formulation of PLGA exenatide microspheres were prepared following single-emulsion solvent evaporation method by Kwak et al (15) and evaluated the pharmacokinetics and efficacy of the microspheres in both Zucker diabetic fatty and Zucker diabetic fatty lean control rats lowered non-fasting blood glucose and HbA1c concentrations more effectively compared with twice-daily administration of exenatide.

A 1-monthly Exenatide PLGA microsphere whose hypoglycemic efficiency was similar to that of twice-daily exenatide injection was prepared following double-emulsion solvent evaporation method by Liu et al (16). A porous PLGA microsphere which showed SR over 5 days both in vitro and in vivo was fabricated by loading with palmitoyl-acetate to aid exenatide absorption onto the hydrophobic surface of PLGA microsphere for pulmonary delivery by Kim et al (17).

However there were no reports on evaluating the effect of different PLGA microsphere preparation methods on exenatide release and it’s corresponding pharmacokinetics. Therefore in our study we use solvent extraction and co-solvent methods as approaches used to prepare the microspheres, because these processes are simple and convenient to control (11). The use of different organic solvents results in microspheres with various characteristics. Thus, discrepancies of the resultant particles in terms of, for example, release behaviour will be generated. Therefore, the objective of the current study is to

a) To prepare different EX-4 PLGA Microsphere formulations following different approaches
b) To evaluate & compare the invivo pharmacokinetics of different EX-4 PLGA Microsphere formulations
c) To select the best EX-4 PLGA microsphere formulation among the different EX-4 PLGA Microsphere formulations prepared
MATERIALS AND METHODS:

PLGA with a molar ratio of D.L-lactide/glycolide 75/25 (Mw 13 kDa) was purchased from Sigma Aldrich (Bangalore, India). Exenatide was provided by KJD Pharmaceutical Pvt Ltd. (Hyderabad, India). Poly (vinyl alcohol) was provided by Sigma Aldrich (Bangalore, India). SPG membranes were purchased from SPG Technology Co. Ltd. (Miyazaki, Japan). The SPG premix membrane emulsification equipment (FMEM-500 M NERCB, Beijing, PR China). Ethyl acetate, Acetonitrile and trifluoroacetic acid (TFA) (HPLC grade) were purchased from Sigma Aldrich (Bangalore, India). All other reagents were analytical grade.

Preparation of Microspheres:
Exenatide PLGA Microspheres were prepared by following four different approaches such as HS-solvent evaporation, US-solvent evaporation, solvent extraction and the co-solvent methods and are abbreviated as HSE, USE, SoEX and COS respectively. To control particle size, narrow down the size distribution and realize mass production, Shirasu porous glass (SPG) premix membrane emulsification has been employed. So, before preparation, SPG membrane 50.2 µm in size was installed in the equipment.

HSE Microspheres:
The primary emulsion (W1/O) of 1 mL exenatide aqueous solution (3%, w/v, W1) was emulsified with 8 mL methylene chloride as oily phase (O) containing PLGA (10%, w/v) by homogenization (T18, IKA, Germany) at 18,000 rpm for 60 s. Next, the primary emulsion prepared in the step above W1/O was stirred at 250 rpm for 1 min with external aqueous phase (W2) containing PVA (2%, w/v) and NaCl (0.5%, w/v) to form coarse W1/O/W2 emulsions. Then they were poured into premix reservoir and extruded through the SPG membrane (50.2 µm) by N2 pressure to achieve uniform-sized droplets. After that, they were solidified at room temperature for 5 h. Finally, the microspheres were collected by centrifugation, washed with distilled water for 5 times and obtained after freeze-drying.

SoEX Microspheres:
The primary emulsion (W1/O) of 1 ml exenatide aqueous solution (3%, w/v, W1) was emulsified with 8 ml EA as oily phase (O) containing PLGA (10%, w/v) by homogenization (T18, IKA, Germany) at 18,000 rpm for 60 s. Next, the primary emulsion prepared in the step above W1/O was stirred at 250 rpm for 1 min with external aqueous phase (W2) containing PVA (2%, w/v) and NaCl (0.5%, w/v) to form coarse W1/O/W2 emulsions. These were then poured into a premix reservoir and extruded through the SPG membrane by N2 pressure at 5 kPa to achieve uniform-sized droplets. The uniform-sized droplets achieved by extrusion through SPG membrane were poured quickly into solidification solution with a large volume (1.6 L containing 0.9% (w/v) NaCl) under magnetic stirring at 250 rpm for 4 h to solidify the microspheres. The microspheres were obtained in the same way as above.

COS Microspheres:
The exenatide drug in the powder form was dissolved in mixture of organic solvent (MC: MeOH :: 6:2) containing PLGA (10%, w/v). The other steps were same as those for SoEX Microspheres method.

In-vitro Drug Release
PLGA microspheres (10 mg) were dispersed in 1 ml 10 Mm phosphate buffer saline (PBS) medium (pH 7.4) and incubated under agitation at 37°C. At each time interval, supernatants were collected by centrifugation for 3 min at 300 g and replaced with fresh buffer of equal volume.

Drug Analysis
The concentration of exenatide in the supernatant by injecting 5 µL into HPLC. Shimadzu UFLC LC-20ACXR instrument was used with a reverse phase C-18 2.0 x 30 mm, 5 µ column at a flow rate of 0.6mL/min. 0.1% Formic acid in water was used as Mobile Phase A and 0.1% Formic acid in Methanol was used as Mobile Phase B using gradient elution method at 95% B in 5 minutes.

Pharmacokinetics Study
This study was conducted in compliance with Institutional Animal Ethics Committee (IAEC) requirements. The protocol has been approved by the Institutional Animal Ethics Committee (IAEC) of JVR Bio Life Sciences Pvt Ltd. All the ethical
practices as laid down in the CPCSEA guidelines for animal care will be followed during the conduct of the study. The animal experiments were performed at JVR Bio Life Sciences Pvt Ltd (Hyderabad, India).

Male SD rats (8 - 10 weeks & 250–300 g) were divided into six groups (n = 6 per group): Bydureon® Marketed LAR Microsphere Injectable, Exenatide solution, HSE, USE, SoEX and COS groups respectively. The rats in the Bydureon® injectable microsphere LAR Injectable formulation group was dosed subcutaneously at 0.72 mg per rat and exenatide solution group were subcutaneously injected with exenatide at a dose of 36 µg per rat. The other groups were subcutaneously injected with microspheres containing exenatide at a dose of 1 mg per rat (equivalent to the dose of a twice-daily injection of exenatide solution for 2 weeks). 0.25mL of blood samples were collected from retro orbital plexus under anaesthesia at (pre-dose) 0, 0.5, 2, 4, 6, 10, 24, 96, 168, 240, 336, 432, 528, 624 and 720 hr post dose administration. Plasma was obtained by centrifuging blood samples at 3500 rpm for 10 min. under refrigeration (2-4 °C) within 30 minutes of sampling. The obtained plasma samples was separated into a pre-labeled tubes and stored at -70±10 °C until analysis.

**Instrumentation and Chromatographic Conditions**

The UPLC/MS/MS system consisted of an Agilent 1290 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) coupled to an Applied Biosystems Sciex Qtrap 5500 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) using electrospray ionization (ESI). Chromatography was performed on a ZORBAX RRHD Eclipse Plus C18 (1.8 mm, 50 mm, 2.1 mm) maintained at 40°C using a gradient elution with 0.2% formic acid as solvent A and methanol as solvent B. The gradient involved: 10% B for 1.5 min; a linear increase from 10% to 90% B in 1.0 min, 90% B for 0.5 min; a linear decrease from 90% to 10% B in 0.1 min; and equilibration at 10% B for 2.0 min. The flow rate was 0.5 mL/min without a split. Multiple reaction monitoring (MRM) at unit resolution involved transitions of the protonated forms of exenatide at m/z 1047.4-396.3 and of bivalirudin at m/z 1090.7-650.3 in the positive ion mode. Optimized MS conditions were described as follows: curtain gas, gas 1 and gas 2 (all nitrogen) with 35, 55 and 55 units, respectively; dwell time with 100 ms; ion spray voltage with 5500 V; source temperature with 575 1C; declustering potentials with 240 V for exenatide and 100 V for bivalirudin; collision energies with 42 eV (m/z 1047.4-396.3) for exenatide and 48 eV for bivalirudin (m/z 1090.7-650.3).

Sample preparation

All frozen plasma samples were thawed at room temperature and subjected to solid phase extraction (SPE) as follows. An aliquot of 50 µL plasma, 50 µL methanol/Milli-Q water/0.1% formic acid (90:10:0.1, v/v/v) and 50 µL IS solution were added in the 1.5 mL Eppendorf tube. The mixture was vortex-mixed for 1 min and then centrifuged at 15000 g for 5 min. After precondition of 1 mL of methanol and 0.5 mL of formic acid (0.5%) in Milli-Q water, the supernatant was transferred to the Oasis® MCX. The columns were washed with 0.5% formic acid in Milli-Q water, and then methanol/2% aqueous formic acid 96:4 (v/v). The analyte and IS were eluted with two 200 mL portions of acetonitrile/methanol/Milli-Q water/25% aqueous ammonium hydroxide (4:1.1:1.0, v/v/v/v) to 10 mL plastic tube. The collection was added with 200 µL acetonitrile/methanol/Milli-Q water/formic acid (6:5:1.0:1, v/v/v/v), and then evaporated to dryness at 50 °C under a gentle stream of nitrogen. The sample was reconstituted in 50 µL methanol/water/0.1% formic acid (90:10:0.1, v/v/v) and vortexed for 30 s. A 10 µL aliquot of the sample solution was injected into the UPLC/MS/MS system.

Pharmacokinetic Data Analysis:

Based on the individual plasma concentration, pharmacokinetic (PK) parameters was calculated by non-compartmental analysis by using PhoeniX™ WinNonlin® Version 6.4 (Pharsight Corporation, USA). Pharmacokinetic parameters including peak plasma concentration (Cmax), time to reach the peak plasma concentration (Tmax), half-life (T1/2), AUC0-t, AUC0-inf, Vd, CL, and MRT was estimated. The area under the concentration-time curve (AUC) was estimated by linear trapezoidal method and the apparent elimination rate constant (Keq) was calculated by the least squares regression analysis. The cumulative release in vivo was evaluated as follows:

\[
\text{Cumulative release invivo} = \frac{AUC_{0-t} + \frac{C_{246}}{K_{el}}}{AUC_{0-320} + \frac{C_{246}}{K_{el}}}
\]

**RESULTS AND DISCUSSION:**

Uniform-sized PLGA based Exenatide microspheres were prepared by using different methods including solvent extraction (SoEX), Co-Solvent (COS), HS-solvent evaporation (HSE), US-solvent evaporation (USE) and how these methods affects the properties of drug-loaded microspheres, such as encapsulation efficiency.
release behaviour and their pharmacokinetics were evaluated in this study.

The cumulative release profiles in vitro of USE MS and HSE MS were quite different. HSE MS exhibited an initial burst release (~27% within 24 h) followed by an extended drug release over 40 days. However, its release rate leveled off afterwards until reached saturation (about 80%). Conversely, USE MS presented a typical triphasic profile with an initial lower burst (about 14% within 24 h). During a period up to 3 weeks, the release rate was slow, but then the release rate was increased. Finally, the saturation was achieved with uncompleted release (about 75%). SoEX MS showed a plateau with the highest burst (~52%), and after 5 weeks the release rate increased. On the other hand, COS MS exhibited a similar release behavior as SoEX MS but with the slowest burst (~15%).

![Figure 1: Cumulative Mean (±SD) Exenatide release from PLGA microspheres](image)

Mean plasma concentration profile and pharmacokinetic parameters of exenatide following subcutaneous administration of different solution and microsphere formulation is provided in Figure 2 and Table-1.
Dose administered to each group is given in the legend as mg per rat.

Figure 2: Mean Plasma Concentration vs Time Profile of Exenatide following subcutaneous administration of different Solution and Microsphere formulation’s in Male Sprague Dawley Rats (n=6)

Table 1: Mean (± SD) Pharmacokinetic parameters of exenatide following subcutaneous administration of different solution and microsphere formulation(s) in Male Sprague Dawley Rats (n=6)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Dose (mg)</th>
<th>Route</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt; (hr)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (hr)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>AUC&lt;sub&gt;0-4&lt;/sub&gt; (hr*ng/mL)</th>
<th>AUC&lt;sub&gt;0-720&lt;/sub&gt; (hr*ng/mL)</th>
<th>Cumul Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>BYDUREON</td>
<td>0.72</td>
<td>SC</td>
<td>38.7</td>
<td>72</td>
<td>3.51 ± 0.48</td>
<td>972.97 ± 132.29</td>
<td>979.06 ± 133.12</td>
<td>848.31 ± 115.34</td>
</tr>
<tr>
<td>COS MS</td>
<td>1</td>
<td>SC</td>
<td>137.6</td>
<td>0.5</td>
<td>60.56 ± 8.23</td>
<td>9818.98 ± 1335.03</td>
<td>11586.64 ± 1575.36</td>
<td>9818.98 ± 1335.03</td>
</tr>
<tr>
<td>EX-4 Sol</td>
<td>0.036</td>
<td>SC</td>
<td>4.3</td>
<td>2</td>
<td>243.98 ± 33.17</td>
<td>550.56 ± 74.86</td>
<td>554.55 ± 75.4</td>
<td>NC</td>
</tr>
<tr>
<td>HSE MS</td>
<td>1</td>
<td>SC</td>
<td>247.5</td>
<td>2</td>
<td>139.13 ± 18.92</td>
<td>4627.59 ± 629.19</td>
<td>5445.21 ± 740.35</td>
<td>4627.59 ± 629.19</td>
</tr>
<tr>
<td>SoEX MS</td>
<td>1</td>
<td>SC</td>
<td>466.4</td>
<td>2</td>
<td>196.42 ± 26.71</td>
<td>3800.79 ± 516.77</td>
<td>5121.42 ± 696.33</td>
<td>3800.79 ± 516.77</td>
</tr>
<tr>
<td>USE MS</td>
<td>1</td>
<td>SC</td>
<td>318.7</td>
<td>2</td>
<td>29.31 ± 3.99</td>
<td>3751.84 ± 510.11</td>
<td>5587.89 ± 759.75</td>
<td>3751.84 ± 510.11</td>
</tr>
</tbody>
</table>

Table 2: Mean Dose normalized Pharmacokinetic parameters of exenatide following subcutaneous administration of different solution and microsphere formulation(s) in Male Sprague Dawley Rats (n=6)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Dose (mg)</th>
<th>Route</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;/D (ng/mL/mg)</th>
<th>AUC&lt;sub&gt;0-4&lt;/sub&gt;/D (hr*ng/mL/mg)</th>
<th>AUC&lt;sub&gt;0-720&lt;/sub&gt;/D (hr*ng/mL/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BYDUREON</td>
<td>0.72</td>
<td>SC</td>
<td>4.875</td>
<td>1351.347</td>
<td>1359.806</td>
</tr>
<tr>
<td>COS MS</td>
<td>1</td>
<td>SC</td>
<td>60.56</td>
<td>9818.980</td>
<td>11586.64</td>
</tr>
<tr>
<td>HSE MS</td>
<td>1</td>
<td>SC</td>
<td>139.13</td>
<td>4627.590</td>
<td>5445.210</td>
</tr>
<tr>
<td>SoEX MS</td>
<td>1</td>
<td>SC</td>
<td>196.42</td>
<td>3800.790</td>
<td>5121.420</td>
</tr>
<tr>
<td>USE MS</td>
<td>1</td>
<td>SC</td>
<td>29.31</td>
<td>3751.840</td>
<td>5587.890</td>
</tr>
</tbody>
</table>
The peak maximum concentration \( (C_{max}) \) for Bydeuron, COS, HSE, SoEX and USE microspheres was 3.51, 60.56, 243.98, 139.13, 196.42 & 29.31 ng/mL respectively indicating that higher \( C_{max} \) was achieved by HSE microsphere formulations. \( T_{max} \) of Bydeuron was achieved at 72hr, COS microspheres at 0.5hr whereas HSE, SoEX, USE and EX-4 formulations demonstrated similar \( T_{max} \) of 2 hr. The exposures achieved by Bydeuron, COS, EX-4Sol, HSE, SoEX and USE microspheres were 972.97, 9818.98, 550.56, 4627.59, 3800.79 and 3751.840 hr.ng/mL respectively indicating that COS microspheres achieved highest exposure than other formulations. Upon dose normalization and comparing the peak maximum concentrations \( (C_{max}) \) achieved by microsphere formulations with Bydeuron group for COS, HSE, SoEX, USE group was 12.4X, 28.5X, 40.3X and 6X higher whereas the exposure \( (\text{AUC}_0\text{\,day}) \) achieved by microsphere formulations compared with Bydeuron group for COS, HSE, SoEX, USE group was 7.3X, 3.4X, 2.8X and 2.8X higher.

All the microsphere formulations exhibited typical invivo profile of initial burst followed by second peak at later time point. The highest initial burst release of 196.4ng/mL was achieved by SoEX MS formulation it may be due to that when the uniform sized emulsion droplets were added to the solidification solution, they were exposed to volumes of water, resulting in the peptide diffusing near or on the particle surface, whereas the transient second burst of 35.3ng/mL was observed higher for COS MS formulation the reason may be attributed as on incubation the microspheres degraded gradually at an early phase (day 14), the drug distribution was still relatively uniform except for generation of small pores near the surfaces, indicating the drug nearby was released first. At day 30, some drug remained in the core of microspheres; hence, the release rate was very slow in this phase. Afterwards, the drug in the core began to diffuse out of the microspheres resulting in a high release rate. Therefore it was observed that that even though the drug release was controlled by polymer degradation, the internal structural changes of microspheres played the most important role than the decrease of polymer Mw. Our findings adds on to the investigations made by Shi et al who studied how the three different molecular weights of PLGA (10, 20, and 30 kDa,) affects the properties of exenatide microspheres (19). Their results showed that molecular weight and PLGA encapsulation efficiency were directly proportionally related to each other. PLGA with a molecular weight of 30 kDa could achieve a 78.1% ± 8.8% encapsulation rate. 10kDa PLGA microsphere showed a severe initial burst, while the other two microspheres’ release lasted for 30 days similar to the Bydureon microsphere.

The cumulative release of Exenatide from HSE based microspheres was similar to COS MS as in case of HSE MS the reasons may be attributed to non-uniform distribution of the drug droplets as homogenization broke the droplets by agitation with a relatively low shear force, resulting in production of larger non-uniform W1 droplets in the W1/O emulsion. Consequently, large pores were formed in microspheres after solidification the mass loss started rapidly, because its loose inner structure caused more degraded PLGA oligomers and tight-binding drug molecules diffuse out of the matrix easily.

CONCLUSION:
In this study, uniform-sized exenatide-loaded PLGA based microspheres were prepared by SPG premix membrane emulsification. All of the microspheres had a narrow size distribution and an average size of ~20 µm. SoEx MS formulation showed the highest burst release whereas the transient second burst was observed higher for COS MS formulation. As for COS MS, it exhibited a slow release, followed by a fast release in the later phases. The detailed PK based evaluation of PLGA based microspheres prepared by different methods in the study provide help in guiding the emulsion-microsphere preparation or other long-effective release systems , also the results of the study reveals another important point that invirot release behaviours of these microspheres were not influenced by different preparation methods but was affected by internal structure evolution. COS MS was the best formulation due to its constant release rate in vivo over 14 days. Therefore, COS MS can be evaluated further for developing a once-in-a 2 weeks injection of COS MS to replace a BID daily injection of exenatide.

Declaration of conflict of interest:
Harish Kaushik Kotakonda, Malothlu Nagulu, Narasimha Reddy Yellu declare that they don’t have any conflict of interest

REFERENCES:


