Sustained stomach specific delivery of piroxicam from floating emulsion polyelectrolyte complex beads

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This study presents development of floating emulsion polyelectrolyte complex (PEC) beads based on gellan-chitosan PEC and Gelucire 39/01 and 50/13 without using any chemical crosslinker, and release from developed beads of piroxicam (PRM) in 0.1 M HCl (pH 1.2). Developed beads showed excellent buoyancy, significantly improved encapsulation efficiency and sustained release of PRM compared to floating gellan-chitosan beads bearing PRM. Thus emulsion PEC beads may form a potential stomach site specific drug delivery system for PRM delivery.

Keywords: Chitosan, Emulsion PEC beads, Floating drug delivery, Gelucire, Piroxicam

Introduction
Chitosan can form polyelectrolyte complexes (PECs) by electrostatic interaction with polyamions and polysaccharides with COO- or SO4- groups1-5. Beads formed by electrostatic interaction between anionic (COO-) polysaccharide gellan gum (GG) and low molecular weight chitosan (LMCH) in aqueous solutions6 are reported to suffer from poor drug loading due to acidic nature of gelation medium and rapid release in acidic environment of stomach6. Therefore, for better drug entrapment and sustained drug release, in the present study, Gelucires (39/01 and 50/13) were chosen as lipid phase because of their excellent biocompatibility, biodegradability, possession of very low acid (<0.20) and iodine values (<2.0) and prevention of gastric irritation by forming a coating around the drug7-9. Such a drug delivery system is expected to sustain piroxicam (PRM) release in acidic environment of stomach with improved encapsulation efficiency, stability, patient compliance and devoid of disadvantages associated with oil based emulsion gel beads10-13. This study presents development of floating emulsion PEC beads based on gellan-chitosan PEC and Gelucire 39/01 and 50/13, and release study of model drug PRM in 0.1 M HCl (pH 1.2) from developed beads.

Experimental Section
Materials
PRM was gifted by Khandelwal Pharmaceuticals (P) Ltd, Pant Nagar, India. GG (Gelzan™ CM) and LMCH (Brookfield Viscosity 20.00 cps, MW =1500000) were purchased from Sigma-Aldrich (St. Louis, USA), Gelucire 39/01 (waxy solid, m.p. 39°C, HLB = 01) and 50/13 (m.p. 50°C, HLB = 13) was a gift from Gattefosse SAS (St Priest, Cedex, France). Water used was of HPLC grade (Merck) and all other chemicals used were of analytical grade.

Preparation of Floating Gellan-Chitosan (GG-LMCH) PEC Beads
Floating gellan-chitosan PEC beads (P1) were prepared by complex coacervation technique with some modification6. An aqueous solution of GG (1% w/v, with or without PRM) containing CaCO3 (as gas-generating agent) was extruded by 25 ml hypodermic syringe, into LMCH solution (0.6%w/v in 1.5% acetic acid, pH 3.5) at room temperature (RT, 28°C). Beads formed were instantaneously cured for 15 min in gelation medium with mild agitation. Prepared beads were separated by filtration, washed thrice with deionized water and dried in an oven at 40°C for 12 h, and then kept in a desiccator for another 12 h before further experiments. Non floating beads were also prepared for comparison in similar manner but without CaCO3.
Preparation of Gellan-Gelucire Emulsions and Floating Emulsion PEC Beads

Gellan-Gelucire emulsions were prepared by mixing aqueous GG solutions (1% w/v) with Gelucire 39/01 or different mixture of molten Gelucires (39/01 and 50/13) using mechanical stirrer at 500 rpm for 5 min. Floating emulsion PEC beads were prepared by complex coacervation technique with some modification. Emulsions of GG with Gelucire 39/01 or different mixtures of Gelucire 39/01 and 50/13 (10 ml) containing CaCO$_3$ (with or without PRM) were extruded separately by 25 ml hypodermic syringe, into LMCH solutions (0.6%w/v in 1.5% acetic acid, pH 3.5) at RT (28°C). Beads formed were instantaneously cured for 15 min in gelation medium at 37°C with mild agitation. Prepared beads were separated by filtration, washed thrice with deionized water and dried in an oven at 35°C for 12 h, and then kept in a desiccator for another 12 h before further experiments. A total of four emulsion PEC formulations (P2 = 1/0, P3 = 1/0.4, P4 = 1/0.6 and P5 = 1/0.8) were prepared with different Gelucire 39/1: 50/13 ratio.

Microscopic and Scanning Electron Microscopic (SEM) Characterization of Beads

Size of beads was determined with an optical microscope (Model BH-2, Olympus, Japan) fitted with a stage and an ocular micrometer. Dried beads (20) were measured to determine mean diameter of beads. All measurements were in triplicate. Shape, surface morphology and internal structure of dried beads were assessed with a scanning electron microscope (Leo 435VP, variable pressure, Oxford, U.K.) at various magnifications.

Determination of Drug Entrapment Efficiency (EE)

PRM entrapment efficiency (EE) of each formulation was determined by extracting crushed beads with 0.1M HCl (pH 1.2) for 45 min at 37°C and then centrifuged at 5000 rpm. Supernatant layer suitably diluted with 0.1 M HCl, quantifying the amount of drug UV spectrophotometrically at 357 nm. EE = (Actual drug content / Theoretical drug content) x 100.

In vitro Buoyancy of Floating Beads and Drug Release Studies

In vitro study of bead buoyancy and release of PRM from beads was evaluated with a USP XXXI dissolution apparatus type II (paddle type, Electrolab, Mumbai, India) at 50 rpm in 500 ml 0.1M HCl (pH 1.2) at 37±0.5°C. For bead buoyancy, floating beads were separated from submerged beads and their proportion (%) was determined. For drug release, at predetermined intervals, an aliquot (1 ml) was withdrawn and replenished with an equal volume of fresh dissolution medium. Withdrawn samples were analyzed UV spectrophotometrically at 357 nm. In-vitro data were fitted to Higuchi’s square root model$^4$ to analyze kinetics of drug release from beads as $Q = kt^0.5$, where $Q$ is amount of drug released in time $t$, and $K$ is release constant. The data were also subjected to Korsmeyer-Peppa’s power law$^{15}$ as $Mt/M_{\infty} = k^n$, where $Mt/M_{\infty}$ is drug released fraction in time $t$, $K$ is structural and geometric constant, and $n$ is release exponent.

Statistical Analysis

Differences in average of data were compared by analysis of variance (ANOVA one-way) or Student t - test (SigmaPlot® 11). Significance of difference was determined at 95% confident limit (p=0.05).

Results and Discussion

Drug Excipient and Excipient-Excipient Interactions

To study drug-excipient and excipient-excipient interactions, DSC studies on PRM (Heat Flow Endo Up), GG, LMCH, GG-LMCH beads and drug loaded emulsion PEC beads (Heat Flow Endo Down) were carried out. DSC profile of PRM base (Fig. 1a) showed a sharp endothermic peak at 204.87°C corresponding to the melting point of drug. DSC thermogram of pure GG (Fig. 1b) showed a broad endothermic peak at 76.°C corresponding to the loss of water. An exothermic peak observed at 244.9°C indicated degradation of GG. DSC thermogram of pure LMCH sample showed a broad endothermic peak at 71.01° C corresponding to vaporization of water (Fig. 1c). DSC thermogram of GG-LMCH PEC beads exhibited an exothermic peak at 241.5°C (Fig. 1d), which was absent in thermogram of GG and LMCH. Appearance of new exothermic peak at 241.5°C could be attributed to PEC formation. DSC thermogram of emulsion PEC beads (Fig. 1e) bearing PRM exhibited two endothermic and one exothermic peak. First exothermic peak at 40°C could be attributed to melting of Gelucires, whereas, second endothermic peak at 189°C could be attributed to melting of PRM. Shifting of melting endotherm of PRM to lower temperature may not necessarily indicate a potential drug-excipient incompatibility as mixing of drug with excipients lowers the purity of each component in mixture. Exothermic peak at 259°C could be attributed to
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Fig. 1—DSC thermograms of: a) Piroxicam (PRM); b) Gellan gum (GG); c) Low molecular weight chitosan (LMCH); d) Blank floating Gellan-low molecular weight chitosan (LMCH) polyelectrolyte complex (PEC) beads prepared without Gelucires; and e) Floating emulsion PEC beads bearing PRM.
degradation of GG-LMCH polyelectrolyte complex. The data obtained from thermal studies exclude possibility of interaction between drug, Gelucire(s) and polyelectrolyte complex.

Microscopic and Scanning Electron Microscopic (SEM) Characterization of Beads

Diameter of floating PEC beads varied (1.09 ± 0.04 - 1.30 ± 0.02 mm) for different formulations. Floating PEC beads prepared without Gelucire (P1) exhibited smallest bead size. Incorporation of Gelucires resulted in increase in bead size (p<0.05, P2, P3 P4 and P5 compared to P1), may be due to increase in microviscosity of polymeric dispersion, leading eventually to the formation of bigger beads. SEM (Fig. 2) revealed that PRM loaded floating emulsion PEC beads were spherical in shape with rough outer surfaces. Surface of floating emulsion PEC beads, LMCH-polyanion complex, appears as scattered round structures on the surface of beads (Fig. 2b). Surface of GG-LMCH PEC beads clearly showed (Fig. 2d) formation of PEC membrane. Transverse section of floating emulsion PEC beads showed a hollow cavity along with numerous smaller internal pores (Fig. 2c), attributed to the use of gas-generating agent. Upon contact with an acidic medium, CaCO$_3$ effervesced, releasing CO$_2$, which slowly diffused through gel, producing a cross-linked three-dimensional gel network that restricted diffusion of CO$_2$ and resulted in entrapment of released CO$_2$ inside the bead structure, thus producing hollow cavity.$^{16}$

Assessment of in vitro Buoyancy of Floating Beads

Emulsion PEC beads prepared with a gas-generating agent remained buoyant on 0.1 M HCl (pH 1.2) for sufficiently long duration of time (>12 h) with no floating lag time. Upon contact with an acidic medium, CaCO$_3$ effervesced, releasing CO$_2$ and Ca$^{2+}$. Released CO$_2$ was entrapped in gel network producing buoyant formulation and then Ca$^{2+}$ reacted with gellan producing a cross-linked three-dimensional gel network that restricted
further diffusion of CO$_2$, and thus, prolonged floating of polyelectrolyte beads\textsuperscript{16}.

**Drug Entrapment Efficiency (%EE) of Floating Beads**

PRM EE of prepared floating emulsion PEC beads varied (93-98%). PRM is a highly hydrophobic\textsuperscript{17} drug (log $P$ = 3.0, water solubility = 23 mg/l). So it was expected that PRM EE of both floating PEC and emulsion PEC beads would be high. In case of floating PEC beads, high EE could be attributed to entrapment of water insoluble drug in a highly ordered, compact PEC matrix as PECs are reported to exhibit a very high degree of ordering and crystal like properties, and have quite compact structures\textsuperscript{3-5}. In case of floating emulsion PEC beads, during bead preparation, PRM was mixed in a highly viscous emulsion, which when extruded in acidic gelation medium, prevents escape of PRM from beads.

**In vitro Drug Release**

Under *in vitro* drug release profiles (Fig. 3) of PRM from floating PEC and emulsion PEC bead formulations (±SD, $n=3$), from floating PEC beads (P1), 10, 49, 76 and 98 % of PRM was released at the end of 1$^{\text{st}}$, 5$^{\text{th}}$, 9$^{\text{th}}$ and 12$^{\text{th}}$ h respectively. Retardation in drug release could be due to poor solubility of PRM in acidic dissolution medium. Incorporation of Gelucire 50/13 to PEC formulation (P2) resulted in significant difference (p<0.0001) in PRM release with 22, 50, 73 and 99 % of PRM released at the end of 1$^{\text{st}}$, 5$^{\text{th}}$, 8$^{\text{th}}$ and 11$^{\text{th}}$ h respectively. This release pattern was significantly different (p<0.004) from formulation P2. Further increase in Gelucire 39/01 concentration (P5) resulted in PRM release profile, which was significantly different (p<0.014) from formulation P3 and P2 (p<0.002), with 21, 55, 75 and 98 % PRM released at the end of 1$^{\text{st}}$, 5$^{\text{th}}$, 7$^{\text{th}}$ and 10$^{\text{th}}$ h respectively. At highest Gelucire concentration (P5), PRM release was significantly retarded compared to P4 (p<0.0001), P3 (p<0.003) and P2 (p<0.0001).

**Release Mechanism**

*In vitro* release pattern of various formulations was analyzed by fitting dissolution data into various kinetic models (Table 1). For formulations P1, P3, P4 and P5, $r^2$ values were found higher when fitted to Zero order model, which describes that drug release rate from formulations is independent of drug concentration. In case of formulation P2, $r^2$ values were higher when fitted to Higuchi model, which describes release from system, where solid drug is dispersed in an insoluble matrix, and rate of drug release is related to the rate of drug diffusion. The $n$ values indicated that, formulations P1 and P2 followed non-Fickian anomalous diffusion. On the other hand, formulations P3, P4 and P5 followed Fickian and quasi Fickian diffusion respectively.
Conclusions

Drug release was modulated from emulsion gel beads by changing Gelucire 39/01:50/13 ratio. This modification also leads to improved/altered drug entrapment and may also improve drug stability in emulsion gel beads as both PECs and Gelucires improve stability of entrapped drugs because of their excellent physicochemical properties. Developed floating emulsion PEC beads have potential to be used as carrier for oral stomach specific delivery of NSAID’s for optimal therapy to improve efficacy, safety (reduced gastric irritation due to entrapment of drug in emulsion PEC beads, thereby preventing direct contact of PRM with gastric mucosa) and patient compliance, more specifically for geriatric population.

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