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Optimization of formulation parameters for controlled drug delivery from Metformin Hydrochloride loaded Chitosan/TPP microspheres

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Abstract
Diabetes contributes to a major health burden on humans and India has emerged as Diabetes capital of the world. Due to extensive research and demand for novel therapeutics, many agents have been formulated to mitigate disease and improve patient health, one of which is Metformin. This drug has good performance but controlled drug release formulations are required to improve patient compliance and drug bioavailability. We aimed to formulate controlled drug release system using Chitosan/TPP mixture and study their behavior in buffered gastrointestinal conditions. Different parameters such as percentage of Chitosan, TPP, drug and varied stirring time were used to prepare different batches.
These batches were checked for best controlled drug release characteristics in buffered gastrointestinal conditions. High level of sustained drug release was observed in a batch which also has good morphology, drug encapsulation and equilibrium swelling characteristics. As all the trials have been conducted at room temperature, we present a tunable controlled drug release system, showing Metformin as a model drug, for which there is high industrial scalability.

Keywords: Metformin Hydrochloride, Controlled drug release, Chitosan, Sodium tripolyphosphate (TPP).

Introduction
Diabetes is one of the most common and major problem throughout the world and especially Indians are genetically more prone to it (Kaveeshwar & Cornwall, 2014) [8]. A lot of genetic and physiological studies have been done to unravel disease characteristics and its mitigation (Sharma *et al.,* 2014 [6]; Vats *et al.,* 2015) [15]. Apart from prevention of this disease, a lot has been done to control elevated level of glucose in blood by use of many anti-diabetic drugs. Out of these, metformin hydrochloride is the preferred one over other drugs as it is an anti-hyperglycemic drug instead of usual anti-glycemic drugs. This way it controls blood glucose level in diabetic patients and poses lower risk of hypoglycemia due to accidental overdose of drug. However, in spite of favorable patient response and lesser immediate side effects than other drugs, it suffers from some drawbacks such as high dosage needs (1.5 - 2 g/day), low range of oral bioavailability (50-60%) and risks to those with kidney malfunctions. Also in case of traditional administration routes (tablets/injections), there are undesirable drug levels in the blood (Standards of Care for Diabetes, 2009). Upon administration via injections, tablets, etc, there is a sudden discharge of drugs, resulting in patient suffering due to drug side-effects and after a short interval low drug bioavailability, insufficient drug-level, and many more which are quite common in case of traditional formulations.

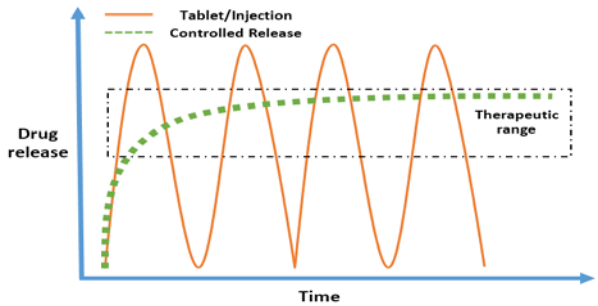

Fig 1: Comparison between traditional (tablet/injection) and controlled release systems

To overcome these problems, many approaches have been undertaken to formulate controlled release systems for its optimal release at desired levels (in therapeutic range) in the blood. Different formulations have been proposed using food grade gelling agents such as Alginate, Carrageenan, etc. In this regard, Chitosan-TPP microspheres have been found to be suitable for controlled release formulations due to its good biocompatibility and biodegradability (Illum, 1998 [10]; Nair *et al.*, 2009 [16]; Balau *et al.*, 2004) [9].

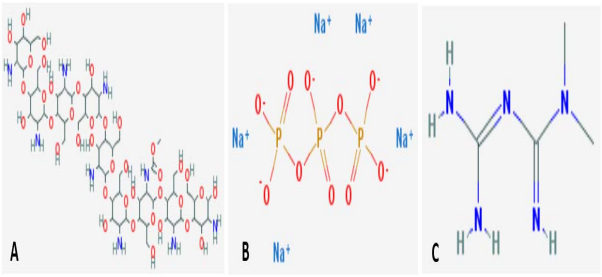

Fig 2: A) Chitosan (Deacetylated), **B)** Sodium Tripolyphosphate **C)** Metformin Hydrochloride

Chitosan microspheres can be prepared by various methods like ionic cross-linking, solvent precipitation, coacervation, modified emulsification and ionotropic gelation, and thermal cross-linking (Dhawan *et al.,* 2004[19]; Dubey *et al.,* 2004) [17]. Out of these we have used ionic cross-linking method having TPP (Sodium tripolyphosphate) as the cross linking agent. Inter or intramolecular links between chitosan (positively charged) and counter-ions of TPP (negatively charged) are formed and they can prove to be a good controlled release formulation.

Drug release and holding capacity of the microspheres is affected by pH of TPP solution, TPP concentration, concentration of chitosan solution, chitosan - TPP interaction time, drug concentration. Thus, the objectives of the present study were to study effect of varying parameters related to chitosan, TPP and drug and to formulate microspheres with a controlled release behavior. Metformin hydrochloride ($C_4H_{11}N_5$) was chosen as the model drug, which is an anti-diabetic drug, and Chitosan-TPP combinations were chosen to prepare ionic cross-linking based microspheres.

2. Materials and Methods

2.1 Materials

Chitosan ($C_{12}H_{24}N_2O_9$, 85% de-acetylated) was procured from Sigma aldrich Co (St. Louis, USA) and Sodium Tripolyphosphate (TPP) was purchased from Loba Chemie Pvt Ltd. (Mumbai, India). The drug 'Metformin hydrochloride' was received as a gift sample from Ranbaxy Laboratories Pvt. Ltd. (Gurgaon, India).

2.2 Preparation of Microspheres

The microspheres were prepared by ionic cross-linking method, described in previous works (Bhumkar & Pokharkar, 2006; [3], Goncalves *et al.,* 2005 [21]; Patel *et al.*, 2006) [1] with some modifications. A chitosan-drug solution (1.5% w/v; 1% w/v) concentration was prepared and microspheres were prepared by dropping this bubble-free solution using a disposable syringe (10 ml) into a gently agitated (rpm < 400) TPP (2% w/v) solution. The microspheres made were separated after certain intervals and rinsed with distilled water. Then, they were air dried for about 24 hrs and kept for oven drying at 37°C for 3 hrs to remove adherent moisture.

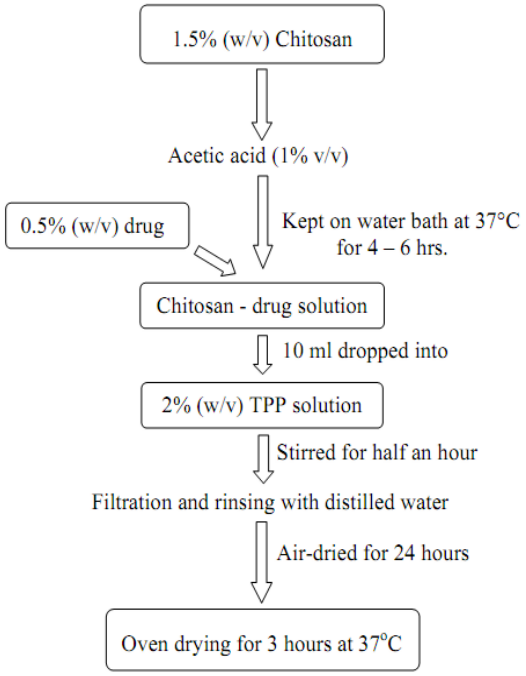

Fig 3: Preparation of Metformin hydrochloride loaded Chitosan-TPP microspheres.

2.3 Percentage yield

After oven drying, the microspheres were weighed and % yield was calculated by dividing it with the total amount of non-volatile components used in the process, as follows:

% yield (w/w) = (wt. of dried microspheres / wt. of Chitosan + wt. of TPP) x 100

2.4 Particle size analysis

Microspheres of varying sizes (800-1600µm) were obtained. Size of the microspheres was observed under Stereomicroscope (Nikon, Japan) in freeze frame at 6.3X magnification.

2.5 Encapsulation efficiency

The microspheres were crushed to powder using mortar - pestle and 50 mg of the powder was taken for calculation of amount of drug actually present in microspheres. This powder was kept for digestion for 24 hrs in a flask containing 10 ml of 0.1 N HCl (Roy *et al.* [18], 2009; Ko *et al.* 2002) [5]. Afterwards, the solution was filtered with Whatman filter paper no. 4 and optical density of the filtrate was taken spectrophotometrically (Hitaichi U2900). Then encapsulation efficiency was calculated using following equation:

% Encapsulation efficiency = (Actual entrapment level / Theoretical entrapment level) x 100

2.6 Equilibrium swelling studies

To evaluate the equilibrium swelling characteristics in buffered gastrointestinal environment, 50 mg of the microspheres were put in different buffers (pH 1.2 and 6.8) at 37°C until they reach the point after which they do not swell anymore (Roy *et al.,* 2009 [18]; Oliveira *et al.*, 2005) [2]. Then, the microspheres were filtered, blotted for drying, weighed and assessed based on following equation:

% Equilibrium swelling =
(wt. at equilibrium - initial wt of microspheres / initial wt of microspheres) x 100

This study was done to see the effect on microspheres when administered into the human body. This also gives an indication of drug release behavior from the microspheres in the gastrointestinal tract.

2.7 Cumulative Drug release study

50 mg of the microspheres were taken to study drug release from the microspheres in buffered gastrointestinal environment. The microspheres were dispensed in 150 ml of pH 1.2 buffer in a conical flask for first 4 hrs and then in 150 ml of pH 6.8 buffer for next 20 hrs under continuous stirring at 37^0C and 250 rpm (Gupta *et al.,* 2001[7]; Chen *et al.,* 2008; [4] Patel *et al.,* 2006) [1]. 3 ml each of the dispensing solution was sampled out to spectrophotometrically (Hitaichi U2900) determine the drug released at ½, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 24 hrs and simultaneously the flask was replaced with equal amount of fresh buffer. The cumulative drug release from the microspheres was estimated by comparing each observation with the drug actually entrapped.

3. Results & Discussion

3.1 Effect of Chitosan/TPP concentration, pH of TPP solution and Stirring time

Various combinations of chitosan and TPP concentration were used for preparation of fully spherical microspheres. Percentage yield which is indicative of the weight gained by the Chitosan solution inside TPP solution in form of microspheres was found to vary considerably with pH and conc. of TPP solution, but doesn't change significantly with different stirring time. This was evident as no distinct microspheres were formed with TPP concentration of 1% and slurry of CTS solution was obtained in case of 3% TPP concentration. The microspheres formed with chitosan concentration lower or higher than 1.5% were either fragile or were of distorted shape. The maximum yield (11.80%) was observed with 2% TPP solution and at pH 7.

Out of all the varying parameters, the microspheres obtained were spherical in case of TPP solution of pH 5, 6, 7 and were distorted in case of pH 3 and 4. Microspheres were also having irregular shapes in case of pH 8 and 9. These are shown in Figure 4 (A - G)

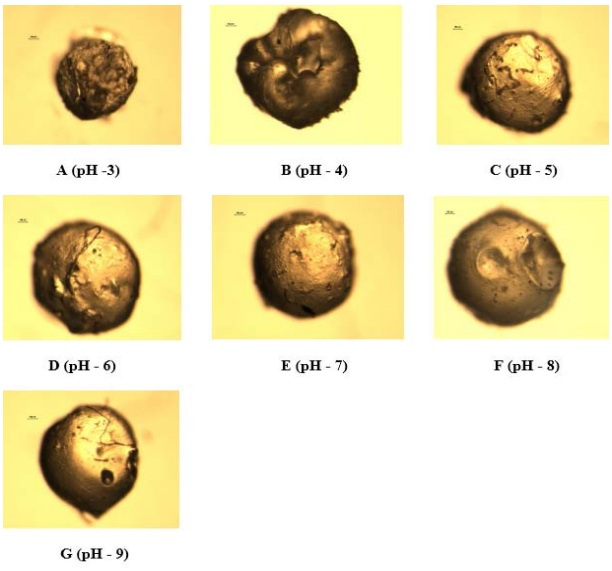

Fig 4: Stereo-microphotographs of chitosan microspheres made with TPP solution at different pH.

Average size of microspheres was calculated and maximum average size observed was 1480 µm in batch containing microspheres made from TPP solution (pH 7).

The TPP solution left out after the formation of microspheres in TPP solution of pH 8, was very hazy due to dissolution of chitosan particles in it. Microspheres formed in other TPP solutions did not dissolve. Microspheres made in TPP solution of pH 3, 4, 5 and 8 were very sticky, their spherical shape was lost during 24 hrs air drying and many of the microspheres got flattened.

Encapsulation efficiencies of batches with different parameters were calculated. The batch made with pH 7 TPP solution was found to have highest encapsulation efficiency (83%).

Equilibrium swelling of different batches in pH buffer 1.2 with different parameters was checked and optimal swelling (346 %) in pH buffer 1.2 was observed in batch made with pH 7 TPP solution.

3.2 Effect of Drug concentration

After above results, batch with TPP concentration of 2% and pH 7, Chitosan concentration of 1.5 % and 30 minutes stirring time was fixed to prepare microspheres and further optimization (drug concentration in Chitosan solution) was undertaken. The percentage yield observed with changed drug concentration was also calculated.

The TPP solution left out after the formation of microspheres in different drug concentration was clear. Microspheres formed in drug concentrations of 0.5 % and 1% were fully spherical but those of 1.5 and 2 % were of irregular shape. Differences in shape of microspheres due to change in drug concentrations have been shown in Figure 5 (A-D).

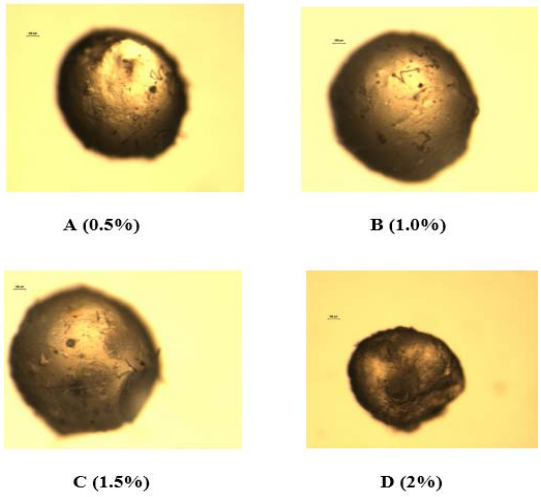

Fig 5: Stereo-microphotographs of chitosan microspheres made with different drug concentration.

Average size of microspheres observed with varying drug concentration was observed to fall in the range of 1200 – 1500 µm with highest being in drug concentration of 0.5 %. Encapsulation efficiencies of batches with different drug concentrations were calculated and the one with drug concentration of 0.5 % was found to have highest encapsulation efficiency.

In terms of equilibrium swelling, there was little difference between swelling of batch with 0.5% and 1% drug concentration in pH buffer 1.2. Maximum swelling in pH buffer 1.2 was observed in batch made with 0.5 % drug.

3.3 Effect of various parameters on *in vitro* cumulative drug release

A general trend observed was that the microspheres swelled in first few hrs and became transparent when put in buffer 6.8 solution in 5th hr. In most of the batches, general trend seen was that there was a drastic release of drug (11 – 26 % difference b/w 3rd and 4th hr and 15 – 19 % difference b/w 4 hrs and 5 hrs O.D.) which may be due to change of pH buffer 1.2 to 6.8 buffer. In most of the batches, after 5th hr the drug release doesn't increase much which may be due to slow dissolution and adaptation of microspheres in the buffer 6.8 solution, after initial burst. As evident in the Figure 6, the microspheres released the drug almost completely in the buffer after 8-10 hrs. Drug release was slower in batch run with more stirring time (45 and 60 mins) and final drug release was even lower. This behavior might be due to increased interaction (cross-linking) between TPP and Chitosan pertaining to more exposure time.

Increase in Chitosan solution volume leads to higher initial drug release and faster release after 1.5 hrs but final release was comparatively lower with the best batch observed.

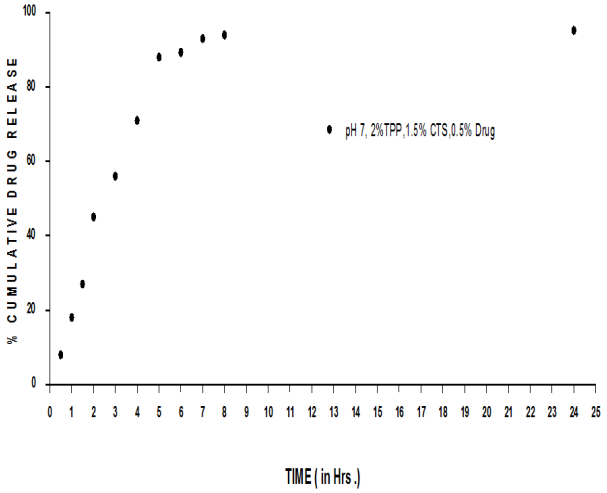

Fig 6: Cumulative drug release from Microspheres (2% TPP, pH 7, 1.5 % Chitosan, 0.5 % Drug, 30 mins stirring.

4. Conclusion

There are a myriad techniques available for entrapment and controlled release of important drugs, but only a few pass all the requirements laid down for safe, effective and targeted drug delivery to humans. Chitosan is a very attractive alternative for use in human system because it has long been used as food additive. Controlled drug delivery systems aim to ensure sustained release of drugs in their therapeutic range and chitosan based microspheres are being increasingly used.

In case of Chitosan/TPP based controlled drug release preparations, Chitosan and TPP concentration, pH of TPP and drug concentration are very important parameters for formation of non-fragile, spherical microspheres with good drug encapsulation and tunable *in vitro* drug release behavior. The difference due to varying stirring time was not much evident upto 60 mins and 30 minutes was found to be enough for proper microsphere formation. Microspheres were of good form when made under stirring speed less than 400 rpm. Thus, Chitosan – TPP based release formulations are an attractive alternative to traditional drug delivery systems.

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