FORMULATION AND PERFORMANCE EVALUATION OF BETAHISTINE DIHYDROCHLORIDE MICROSPHERES AS SUSTAINED RELEASE SYSTEMS

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ABSTRACT
Betahistine dihydrochloride is a histamine-like drug widely used in relieving the symptoms associated with Ménière’s syndrome. Pharmacokinetic studies of betahistine have demonstrated that it has a short plasma half-life of 3-4 hours. In such cases frequent administration of the drug is required in order to keep plasma concentration within the therapeutic range. However, this may lead to noncompliance and aggravate patients’ comfort. An advanced approach for achieving sustained release of drugs is their incorporation in microparticulate carriers. Aim: To design a sustained release microsphere formulation of betahistine providing reduced dose frequency and lower risk of side effects occurrence. Materials and methods: Betahistine-loaded chitosan microspheres were obtained via W/O emulsion solvent evaporation technique and were characterized for particle size, drug loading and entrapment efficiency. Drug release into phosphate buffer saline pH 7.4 was performed and dissolution profiles of the formulations were obtained. To study the mechanism of drug release from the microspheres the dissolution data was fitted to various mathematic models. Results: Betahistine-loaded microspheres were produced with a high drug loading and entrapment efficiency. The microcarriers were spherical in shape with mean particle size of 3.82 μm to 7.69 μm. Betahistine release studies from the microspheres showed similar and slightly increasing dissolution profiles. The drug release proceeded in a controlled manner following Fickian diffusion. Conclusion: The obtained results suggest that betahistine-loaded chitosan microspheres prepared by solvent evaporation method are capable of sustained release of drugs and therefore can be used as drug delivery systems in the treatment of Ménière’s syndrome.

Key words: betahistine dihydrochloride, sustained release, microspheres, solvent evaporation, chitosan

РЕЗЮМЕ
Бетагистин дигидрохлорид представляет собой гистаминовый аналог, широко применяемый для облегчения симптомов, сопутствующих синдром Менière. По данным фармакокинетических исследований бетагистамина имеет краткую плазменную полужизнь – 3.4 ч. В таких случаях, чтобы поддерживать концентрацию плазмы во время терапевтического „окна“, приходится вводить лекарственное вещество часто, через небольшие интервалы. Это может привести к отсутствию содействия при проведении лечения со стороны пациента, как и к ухудшению его комфорта. Современный подход достижения удлиненного освобождения лекарства заключается в его включении в частицы-носители.
ЦЕЛЬ: Разработать лекарственно-доставляющую систему с удлиненным освобождением бетагистина, что способствовало бы уменьшению частоты приема и снижению риска появления нежелательных лекарственных реакций. МАТЕРИАЛЫ И МЕТОДЫ: Микросферы получены через W/O эмульсионную технику с испарением растворителя и охарактеризованы по отношению к их размеру, количественному содержанию бетагистина и эффективности нагрузки. Освобождение лекарственного вещества из носителя проведено в изотоническом фосфатном буфере pH 7.4 и получены профили растворения бетагистина от различных моделей. В целях выяснения механизма освобождения лекарственного вещества из микросфер данные теста растворимости фитированы к различным математическим моделям. РЕЗУЛЬТАТЫ: Получены частицы с высоким содержанием бетагистина и с большой эффективностью нагрузки. Микросферы имели сферичную форму и средний диаметр частиц от 3.82 μм до 7.69 μм. Профили растворения бетагистина различных моделей подобны, освобождение протекает контролируемо, следуя Фиккову диффузию.
ЗАКЛЮЧЕНИЕ: Полученные результаты показывают, что полимерные микросферы
INTRODUCTION

Betahistine dihydrochloride (BET) is a histamine analogue currently prescribed for symptomatic treatment of vertigo, motion sickness, tinnitus and other vestibular disorders of central and peripheral origin. BET improves the microcirculation of the inner ear resulting in reduced endolymphatic pressure in the labyrinth and is, therefore, widely used to relieve the symptoms associated with Ménière’s syndrome.1

In clinical practice, BET is generally administered orally at a dose range 24 to 48 mg/day divided into three to four doses daily. Orally administered doses of BET are rapidly and completely absorbed from the gastrointestinal tract. The drug is quickly metabolized to one primary metabolite - 2-pyridylacetic acid and excreted in the urine.2 Studies with radio-labelled BET have demonstrated a plasma half life of 3-4 hours which necessitates frequent administration of the drug and may lead to noncompliance, especially in elderly patients.3

It was thus advantageous to be able to design a sustained release formulation of BET which allows a reduction of the frequency of dose while keeping drug concentration within the therapeutic range. Eliminating the risk of intolerance and side effects manifestation due to immediate drug release which is common for conventional formulations will improve patients’ comfort and will stimulate their compliance. The permanent relief of the symptoms is expected to improve patients’ quality of life. Since the drug is freely soluble in water, specific technological approaches are required to control drug release.4,6

Modern strategy for achieving sustained release is drug inclusion in polymeric matrix systems. This approach has another important advantage – protection of the substance from adverse environmental conditions. Since BET is highly hygroscopic, its inclusion in the carrier particles will greatly enhance its physical stability.

To produce microparticles with certain physicochemical and morphological parameters, and proper release profile remains a challenge. It is necessary to investigate the influence of numerous variables such as preparation technique or equipment and also optimize the process parameters. The results could serve as a guide for in-depth studies of the microspheres as drug delivery systems for BET.

AIM

The aim of our study was to formulate microparticulate drug delivery system based on chitosan and to study its biopharmaceutical behaviour.

MATERIALS AND METHODS

Betahistine dihydrochloride, chitosan (from shrimp shells, low viscosity, degree of deacetylation >75%), sorbitan monooleate 80 (Span 80) and petroleum ether were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). All other reagents were of analytical grade and were used as provided.

PREPARATION OF CHITOSAN MICROSPHERES

Chitosan microspheres were prepared by emulsion/solvent evaporation technique using liquid paraffin as external phase. Chitosan was dissolved in deionized water containing 2% v/v acetic acid. Accurately weighed amount of BET was added to the polymer solution until a homogeneous solution was obtained. To form a single W/O emulsion, drug-polymer solution was slowly added dropwise to 100 ml preheated liquid paraffin containing 0.5% w/v Span 80 as an emulsifying agent at a constant stirring rate of 1100 rpm for 3.5 hours using a two blade stirrer ES (Velp Scientifica, Usmate, Italy). The temperature was maintained at 65 °C throughout the process which helps in evaporation of the aqueous acidic phase and solidification of the microspheres. The hardened microspheres were separated by vacuum filtration (Nylon 66 membrane filter, 0.45 μm, Sigma) and washed several times with petroleum ether to remove oil. Finally, microspheres were air dried for 24 hrs and then stored in vacuum desiccator for further use. To study the influence of different formulation variables on chitosan microspheres, four batches labeled respectively M1 to M4 were prepared by varying BET and chitosan concentrations and drug/polymer ratio as shown in Table 1.
MICROSPHERE CHARACTERIZATION

1. Drug content and entrapment efficiency

The actual drug content of all the formulations was determined spectrophotometrically. BET-loaded microspheres were dispersed in 20 ml 2 % v/v aqueous acetic acid solution by agitation in ultrasonicator (Siel UST7.8-200, Gabrovo, Bulgaria) for 30 minutes to dissolve the polymer and extract the drug. After filtration, drug concentration was determined after proper dilution using an Ultrospec 3300 pro UV/Visible Spectrophotometer (Biochrom Ltd., Cambridge, UK) at a wavelength of 261 nm. The Drug Entrapment Efficiency (DEE) was calculated according to the following equation:

\[ \text{DEE} \% = \left( \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \right) \times 100 \]

The drug entrapment efficiency for the formulations is reported in Table 1.

2. Particle size analysis

All the batches were studied for shape and size using optical microscope (Eclipse 80i, Nikon Engineering Co. Ltd., Japan) equipped with a camera (DS-Qi1) and computer controlled image analysis software (NIS-Elements, Nikon, Japan). At least two hundred microspheres were measured randomly and the average particle size was determined using the Edmondson’s equation:

\[ D_{\text{mean}} = \frac{\sum_{i=1}^{N} d_i}{N} \]

where \( N \) = number of microspheres observed and \( d \) = mean size range.

3. In vitro drug release study

In vitro release of BET from the microparticles was studied by diffusion with a dialysis bag. The dialysis membrane (Sigma, MWCO 12000 Da) was cut into equal pieces (6x2.5 cm) and soaked in distilled water for 24 hrs before use. An accurately weighed quantity of microparticles (equivalent to 10 mg BET) was suspended in 1 mL of PBS (pH 7.4) and placed in the dialysis bag with the two ends fixed by thread. The bag was attached to the paddles of a USP type II dissolution tester (AT7 Sotax, Allschwil, Switzerland) and put into 500 mL PBS (pH 7.4) dissolution medium. The rotation speed was set at 50 rpm and the temperature of the dissolution medium was maintained at 37 ± 0.5°C. Samples of 1 mL were withdrawn from receptor compartment at regular time intervals and replaced with the same amount of fresh PBS. The samples were then analyzed spectrophotometrically at \( \lambda = 261 \) nm. The in vitro release study was performed in triplicate for each sample.

To study the mechanism of drug release from the microsphere carriers the data obtained in this study was fitted to different kinetic models.

STATISTICAL ANALYSIS

All experiments were repeated at least three times. Results are expressed as means ± SD. Statistical analysis was performed using one-way ANOVA followed by studentized range test using the SPSS Statistics 11.5. A p value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

PREPARATION OF THE MICROSPHERES

Four samples of drug-loaded microspheres were prepared using different concentrations of BET and chitosan (1.0%, 1.5% and 2.0% w/v) and varying drug/polymer ratio (1:1, 1:1.5 and 1:2) to investigate modifications of the particle size, drug entrapment efficiency and release behaviour.

Table 1. Composition and physicochemical properties of BET loaded microspheres

<table>
<thead>
<tr>
<th>Model</th>
<th>Drug concentration %</th>
<th>Polymer concentration %</th>
<th>Drug/polymer ratio</th>
<th>Entrapment efficiency (% ± SD)*</th>
<th>Mean particle size (μm ± SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>1</td>
<td>1</td>
<td>1:1</td>
<td>69.37 ± 0.91</td>
<td>3.82 ± 0.14</td>
</tr>
<tr>
<td>M2</td>
<td>1</td>
<td>1.5</td>
<td>1:1.5</td>
<td>93.02 ± 0.98</td>
<td>4.49 ± 0.24</td>
</tr>
<tr>
<td>M3</td>
<td>1</td>
<td>2</td>
<td>1:2</td>
<td>93.85 ± 2.51</td>
<td>4.52 ± 0.33</td>
</tr>
<tr>
<td>M4</td>
<td>2</td>
<td>2</td>
<td>1:1</td>
<td>98.27 ± 0.73</td>
<td>7.69 ± 0.33</td>
</tr>
</tbody>
</table>

*n = 3.
ENTRAPMENT EFFICIENCY

BET loaded microspheres were produced with a high drug entrapment efficiency. Higher polymer concentration in the emulsion droplets led to an enhancement of the efficiency of BET entrapment from 69.37% to 98.27%. Probably, the higher viscosity of chitosan solution tended to restrict diffusion of the drug in the surroundings and enhanced the drug entrapment efficiency.\(^8\)

OPTICAL MICROSCOPY

Optical microscopy of BET-loaded microparticles revealed spherical geometry. The particle size of each formulation is reported in Table 1. Mean sizes of the formulations ranged from 3.82 to 7.69 μm. Increase in chitosan concentration in a fixed volume of aqueous phase resulted in larger particle diameter. Higher concentration of polymer in the sample led to an enhanced frequency of collisions, resulted in

![Photomicrographs of betaistine-loaded formulations M1 (A), M2 (B), M3 (C) and M4 (D) at 400x magnification.](image)

**Figure 1.** Photomicrographs of betaistine-loaded formulations M1 (A), M2 (B), M3 (C) and M4 (D) at 400x magnification.
The fusion of semiformed particles, and finally increased the size of the microspheres. Photomicrographs of the formulations are presented in Fig. 1.

**In vitro release studies**

The dissolution profiles of BET-loaded formulations are shown in Fig. 2. Dissolution profiles show that BET was released from the microspheres in a biphasic way with initial rapid drug release (burst effect) from the surfaces of the particles followed by a step of slower release. The initial burst release ended within the first 30 - 60 minutes and was probably due to the accumulation of high drug amounts in the periphery of the microspheres during the intense solvent elimination and might be attributed as a desired effect to ensure the initial therapeutic plasma concentrations of drug. The drug in the core of particles is responsible for the prolonged drug release from the polymer matrix. Two phenomena can contribute to enhancing the diffusion of the remaining dispersed drug into the microsphere matrix – formation of pores within the matrix due to the initial drug dissolution and particle wetting and swelling which enhances the polymer permeability to the drug. The release of drug from the polymer microparticles was controlled by the formation of a gel which slowed diffusion of the drug across the viscous boundary layer. It is reasonable to suggest that the prolonged release was the result of the gel formation.

The release patterns of the samples were similar, with slight differences depending on the formulations drug loading and entrapment efficiency. No significant differences (p > 0.05) were found between formulations M1 and M2 and also between M3 and M4 at each data point.

At the end of 10 hrs, release of BET was incomplete (in the range of 56.08% to 64.98%) in all the batches indicating that the chitosan gel layers were too swollen and viscous and hindered the outward transport of core located drug molecules.

To find out the mechanism of drug release after the initial ‘burst effect’, the obtained data was fitted in different kinetic models:

- Cumulative percent drug released vs. Time - zero order kinetics (Fig. 3);
- Log cumulative percent drug retained vs. Time - first order kinetics (Fig. 4);
- Cumulative percent released vs. √T-Higuchi’s classical diffusion equation (Higuchi matrix), (Fig. 5);
- Log of cumulative percent drug released vs. Log time - Korsmeyer-Peppas exponential equation (Fig. 6);
- (Percentage retained drug) 1/3 vs. Time - Hixson-Crowell erosion equation (Fig. 7).

The coefficients of determination for the different drug release kinetic models are shown in Table 2. Models with the highest coefficients of determination were judged to be the most appropriate for the in vitro release study. For all the formulations prepared the best fitting linear parameter was Korsmeyer-Peppas model with coefficients of determination in the range 0.920 to 0.965. Since this model is generally used to analyze the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved, we expected similar patterns. For these cases, a general equation can be used:

\[ \frac{M_t}{M_\infty} = K t^n \]

where \(\frac{M_t}{M_\infty}\) is the fraction of drug released at time \(t\), \(K\) is kinetic constant incorporating structural and geometric characteristics of the delivery system, \(n\) is the diffusional exponent and is indicator of the

**Figure 2.** In vitro release profile of chitosan based microsphere formulations. Each data points represents mean ± SD of six measurements.

**Figure 3.** Plot of % cumulative drug release vs. time.
mechanism of drug transport through the polymer. In the case of spherical matrices, $n \leq 0.45$ corresponds to a diffusion control (Fickian release), $0.45 < n \leq 0.89$ to non-Fickian or anomalous, $n = 0.89$ to Case II (relaxational) transport, and $n > 0.89$ to super case II transport. For determination of exponent $n$ the portion of the release curve was used up to $M_t / M_\infty < 0.6$. The $n$ values as shown in Table 2 were in the range of 0.054 to 0.096 indicating that all the prepared formulations followed the Fickian-diffusion controlled mechanism of drug release.

### CONCLUSIONS

In the present study BET-loaded chitosan microspheres were prepared using emulsion solvent evaporation technique. The microspheres were produced with sufficient production yield and high entrapment efficiency. The dissolution profiles were similar and slightly increasing when higher concentration of chitosan was used. BET release from the microspheres was in controlled manner following Fickian diffusion mechanism. These results clearly

### Table 2. Kinetic parameters for different kinetic models of drug release from the formulations

<table>
<thead>
<tr>
<th>Model</th>
<th>Zero order $R^2$</th>
<th>First order $R^2$</th>
<th>Higuchi $R^2$</th>
<th>Korsmeyer-Peppas $R^2$</th>
<th>n</th>
<th>Hixson-Crowell $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>0.904</td>
<td>0.918</td>
<td>0.953</td>
<td>0.965</td>
<td>0.054</td>
<td>0.913</td>
</tr>
<tr>
<td>M2</td>
<td>0.845</td>
<td>0.866</td>
<td>0.921</td>
<td>0.962</td>
<td>0.060</td>
<td>0.859</td>
</tr>
<tr>
<td>M3</td>
<td>0.724</td>
<td>0.769</td>
<td>0.840</td>
<td>0.920</td>
<td>0.094</td>
<td>0.754</td>
</tr>
<tr>
<td>M4</td>
<td>0.773</td>
<td>0.813</td>
<td>0.877</td>
<td>0.943</td>
<td>0.096</td>
<td>0.801</td>
</tr>
</tbody>
</table>

$R^2 =$ coefficient of determination, $n =$ diffusional exponent.
indicate that the chitosan microspheres could be used as a sustained-release drug delivery system.

ACKNOWLEDGEMENTS
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