Formulation, micro- and macrostructural characterization of buccal delivery system, containing vitamin B_{12}

Ph.D. thesis

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Introduction

The common reason of the pernicious anaemia is the acute deficiency of the vitamin B_{12} (cyanocobalamin). Because of the poor oral bioavailability of the vitamin its intramuscular (i.m.) injection form is used frequently in the therapeutic practice. The daily dose of the vitamin B_{12} is only 0.4-2.8 μg and a healthy human liver can accumulate sufficient quantity for two entire years.

The cyanocobalamin has a large molecular weight (M=1355.7 g/mol), but based on clinical studies it can get through the mucous membranes by passive diffusion, which could enable the possible noninvasive applications of the vitamin B_{12}.

Based on its poor bioavailability and good solubility, the B_{12} belongs to the third class of the Biopharmaceutical Classification System (BCS). It means that it can be successfully applied in a dosage form which assures sufficient time for its membrane penetration.

The application of buccal dosage forms has many advantages, as buccal route can be used for systemic delivery because the mucosa has a rich blood supply and it is relatively permeable. This route of drug delivery is of special advantages, including the bypass of first pass effect and the avoidance of presystemic elimination within the GIT. By the use bioadhesive polymeric systems the proper adhesivity to the mucosa can be assured, thus the absorption of the drug through the membrane will be supported.

The applicability of the dosage forms determined by the combination of micro- and the macrostructural properties. The commonly used macrostructural measurements (determination of the mechanical behaviour, dissolution testing)

are well completed by the application of the universal microstructural methods requiring small amount of samples.

Aims

The purposes of my work were the followings:

- Formulation of buccal films containing vitamin B_{12},
- Selection of the proper excipients for the buccal formulation,
- Determination of the optimum proportion of the excipients,
- Looking for correlation between the micro- and macrostructure of the prepared films,
- Examination of the effect of polymer proportion, the way of preparation and the storage on the in vitro drug release profile,
- To prove the drug diffusion through a model membrane.

Methods

Solvent cast and freeze-dried films, containing a water-soluble active agent, the vitamin B_{12} were prepared from two polymers, sodium alginate (SA), and Carbopol 71G (CP).

The ATR-FTIR spectra of the unstored and stored Carbopol powder samples were scanned over wavenumber range of 4000-300 cm^{-1}, at a resolution of 4 cm^{-1} using Able Jasco FT-IR 4200 type A spectrometer with ATR Pro470H single reflection ATR accessory.

Viscosity of the hydrogels was determined by AR 2000 Rotational Rheometer (TA Instruments, New Castle, USA) in a parallel plate configuration.

The upper moved portion was a 40 mm diameter stainless steel plate, the shear rate was 7.409-74.09/s. The pH of the solutions was measured by using ”pH 210 Microprocessor pH Meter” (Hanna Instruments, USA).

For positron annihilation lifetime spectroscopy (PALS) measurements, a positron source made of carrier-free ^{22}NaCl was used. Its activity was around 10^5 Bq and the active material was sealed between two very thin Kapton foils. Lifetime spectra were measured with a fast-fast coincidence system based on BaF_2 detectors and Ortec electronics. All the lifetime spectra of 3600 s measuring time were evaluated individually by the Resolution computer code, the indicated errors are the standard deviations of the lifetime parameters obtained. For Doppler-broadening measurement, a high purity germanium detector (HPGe) was used with Tennelec electronics. The annihilation photo peak contained about 10^6 counts in each case. The energy resolution of the system was around 1.1 keV at 511 keV.

Puncture strength (PS) of films was measured with a texture analyzer (TA.XT® plus Texture Analyser, Stable Micro System Ltd., UK) operating with a 5 kg load cell, two perforated special stainless steel plates of 8 mm hole diameter, and a stainless steel cylinder probe of 5 mm diameter and a texture analyzer (Brookfield LFRACT3-4500, Brookfield Eng. Lab. Inc., USA) operating with a 4.5 kg load cell, Brookfield TA-FS film support fixture (d = 10 mm) and TA-8 stainless steel spherical probe (d = 6.35 mm), respectively. The PS was calculated in both cases using the equation:

$$PS\ [\mathrm{N/mm^2}] = F/A,$$

where F is the load required for puncture, A is the cross-sectional area of the of film.

In vitro dissolution test was performed in a multibath (n=8) dissolution test system Hanson SR8-Plus (Hanson Research, USA). The apparatus was used with paddles at stirring speed of 50 rpm. Each film and wafer was dissolved in 300 ml of pH=6.8 phosphate buffer (Ph. Eur. VII.). Dissolution medium was temperature controlled at 37.0 ± 0.5 °C. 500 µl of samples were withdrawn at 15 minutes regular intervals without replacing by fresh medium. The samples were filtered through 10 µm sample filter (Hanson Research, USA) before further analysis. HPLC analysis was performed by an Agilent 1260 Infinity LC system in conjunction with an Agilent 6460 triple-quadrupole mass spectrometer. The column was a Waters Sunfire C18 (50 mm x 2.1 mm x 3.5 µm) and the mobile phase consisted acetonitrile (10%) and 0.1 M HCOOH in water (90%). The column temperature was 25 °C. The volume of injection was 30 µl and the flow rate 0.5 ml/min. The mass spectrometer, performed with a Jet Stream electrospray ion source (ESI), and the analysis were carried out in positive ion mode. In the MRM mode the precursor and the product ions were 678.5 ($[M/2+H]^+$) and 146.9.

To examine the release mechanism of active ingredient from the prepared cast films and wafers, the results were analyzed according to the following equation

$$\frac{M_t}{M_\infty} = k \cdot t^n,$$

where M_t and M_∞ are the cumulative amount of the released drug at time t and infinite time, k is a kinetic constant depends on structural and geometrical characteristics of the drug-polymer system, n is the transport coefficient related to release mechanism.

Results

- Second order interactions existing between the vitamin B_{12} and the polymers of the buccal films, influencing the stability of dosage forms, were evaluated by the combination of non-invasive microstructural examination methods (FTIR and PALS).

- The applied two methods enabled the selection of the type from the two Carbopol polymers of similar structures which is rapidly wetting and provides sufficient mucoadhesivity [1].
- Homogeneous films were obtained from SA-CP composites of a certain proportion of the components and for its determination real-time PALS method was developed for the first time in the literature [3].
- Micro- and macrostrucrural properties of homogeneous cast and freeze-dried films, which are decisive from the point of application, were studied. I have found that the micro- and macrostructural properties were changed together as a function of the composition.

  The microstructural examination methods confirmed the same basic and different supramolecular structure of the composition showing anomalous macrostructural behaviour.
- Along with the increase of the CP concentration and the storage interval and the use of freeze drying process, the drug release rate was decreased. The changes in the release rates, evaluated by the K&P model, were in good compliance with the results of the microstructural examination methods.

Conclusion

Cast and freeze-dried buccal formulations were prepared from SA and CP of various proportions. The obtained formulations could be suitable for the therapeutic intake of the water-soluble vitamin B_{12}.

Practical relevance of the results:

By tracking the free volume changes, which are invisible for the conventional structural test methods, the PALS and related techniques proved to be unique in each critical step of risk assessment (drug-excipient compatibility, stability) of pharmaceutical development.

The correlation between the supramolecular structural changes of the systems and several pharmaceutically important material characteristics, e.g. compatibility, stability, homogeneity, phase transition, mechanical properties enabled wide variety of uses in almost all phase of pharmaceutical development.

List of original publications

Papers connected to the Ph.D. thesis

1. Szabó B, Süvegh K, Zelkó R. (2011) Effect of storage on microstructural changes of Carbopol polymers tracked by the combination of positron annihilation lifetime spectroscopy and FT-IR spectroscopy. Int J Pharm, 416: 160-163.

2. Szabó B, Hetényi G, Majoros K, Miszori V, Kállai N, Zelkó R. (2011) Bukkális hatóanyag-leadó rendszerek formulálásának és ex vivo vizsgálatának lehetőségei. Acta Pharm Hung, 81: 165-172.

3. Szabó B, Süvegh K, Zelkó R. (2012) Real-time positron annihilation lifetime spectroscopy for the detection of the hydrocolloid gel-film transition of polymers. Polym Test, 31: 546-549.

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5. Kállai N, Sebestyén Z, Szabó B, Simon V, Antal I, Zelkó R. (2012) Intraorális gyógyszerformák fejlesztési lehetőségei. Acta Pharm Hung, 82: 95-104.

Proceedings connected with the Ph.D. thesis

6. Szabó B, Molnár M, Süvegh K, Zelkó R. (2011) Tracking of the microwave induced supramolecular structural changes of polymers with positron annihilation lifetime spectroscopy. Eur J Pharm Sci, 44: (S1) 196-197.

Other publications

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