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Research paper

Determination of dependencies among *in vitro* and *in vivo* properties of prepared mucoadhesive buccal films using multivariate data analysis

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ABSTRACT

Mucoadhesive films represent the most developed medical form of buccal application. Despite the intense focus on buccal film-based systems, there are no standardized methods for their evaluation, which limits the possibility of comparison of obtained data and evaluation of the significance of influence of formulation and process variables on properties of resulting films. The used principal component analysis, together with a partial least squares regression provided a unique insight into the effects of *in vitro* parameters of mucoadhesive buccal films on their *in vivo* properties and into interdependencies among the studied variables. In the present study eight various mucoadhesive buccal films based on mucoadhesive polymers (carmellose, polyethylene oxide) were prepared using a solvent casting method or a method of impregnation, respectively. An ethylcellulose or hydrophobic blend of white beeswax and white petrolatum were used as a backing layer. The addition of polyethylene oxide prolonged the *in vivo* film residence time (from 53.24 ± 5.38–74.18 ± 5.13 min to 71.05 ± 3.15–98.12 ± 1.75 min), and even more when combined with an ethylcellulose backing layer (98.12 ± 1.75 min) and also improved the film's appearance. Tested non-woven textile shortened the *in vivo* film residence time (from 74.18 ± 5.13–98.12 ± 1.75 min to 53.24 ± 5.38–81.00 ± 8.47 min) and generally worsened the film's appearance. Mucoadhesive buccal films with a hydrophobic backing layer were associated with increased frequency of adverse effects.

1. Introduction

Oral mucosa as one of the specific surfaces of the human body is permanently exposed to external factors related to food intake, breathing and speaking processes, which can lead to the onset of some disorders. Oral mucosa diseases affect the majority of the population in the course of their life. Many oral mucosa diseases are manifested by lesions (recurrent aphthous stomatitis, herpetic stomatitis, stomatitis simplex, etc.). The effectiveness and quality of therapy of these diseases could be improved by the application of mucoadhesive buccal films (MBFs) used as dressings which separate the lesion from the environment of the oral cavity. In addition, MBFs may extend the drug's otherwise short exposure time on the oral mucosa and provide a measured dose of the drug at the site of application. MBFs are defined in the monograph of "oromucosal preparations" of the Ph.Eur., but are not explained in detail here. MBFs belong to orodispersible or slowly eroding formulations like orodispersible films but they show adhesive properties in contrast to orodispersible films [1,2]. The ideal MBFs should be flexible, adaptable, comfortable, strong and mechanically durable enough to withstand damage due to stress from mouth activities [3–5].

Due to their advantages (prolonged residence time, providing long periods of therapeutic drug levels at diseased sites, good active ingredients stability) represent mucoadhesive films or patches the most recently developed medical form of buccal application. Films are generally laminates consisting usually of two or more layers, which, thanks to their flexibility and comfortable

Abbreviations: APIs, active pharmaceutical ingredients; AS, taste/aftertaste sensation; BL, backing layer; BS, burning sensation; CFD, cause of film detachment; EC, ethylcellulose; FA, feelings during application; FDM, film detachment mechanism; FIS, feeling of increased salivation; FN, feeling of discomfort in the mouth; FRT, film residence time; FS, feeling a swelling; Gly, glycerol; GPBL, gradual peeling of the backing layer; IT, itching; MBFs, mucoadhesive buccal films; NaCMC, carmellose sodium; PAS, pain sensation; PCA, principal component analysis; PEBL, peeling of the entire backing layer; PEF, peeling of the entire film; PEO, polyethylene oxide; PLS, partial least square regression; PS, pressure sensation; SP, spontaneous; WD, while drinking; WS, while speaking.

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use, are preferred over adhesive tablets. The thinness of the film with non-irritating properties and strong mucoadhesiveness of the polymer demand only minimal changes in the patients' normal activities such as eating, drinking or speaking. Flexible films or patches of various sizes allow their adaptation to the morphology of the oral cavity and size of the defect [6,7].

The most widely used technologies for the formulation of MBFs are the *solvent casting* (*evaporation*) *method* and *hot-melt extrusion*. The *solvent casting method* is based on preparation of a casting solution or suspension, which is subsequently transferred to a casting mold where the solvent evaporates. The final steps are the adjustment of the medical form to the desired size and packaging [4]. *Hot melt extrusion* has been widely used in the pharmaceutical industry to manufacture tablets, granules, and pellets over the last 20 years. Repka et al. investigated the use of hot-melt extrusion for the manufacture of MBFs [8]. Printing of active pharmaceutical ingredients (APIs) onto a base film layer presents a new approach in manufacturing film preparations. Advantages of printed films are given in industrial manufacturing, as APIs are only presented in the printing process [1]. Compressing or freeze-drying of polymer powders and mixtures are alternative methods to manufacture oral film dosage forms [1].

To date, a wide variety of mucoadhesive materials have been used for the development of new pharmaceutical preparations. Mucoadhesive polymers should have certain physico-chemical characteristics including hydrophilicity, visco-elastic properties, flexibility for interpenetration with mucus and epithelial tissue, and numerous hydrogen bond-forming groups such as hydroxylic –OH, carboxylic –COOH, sulfate $-SO_3H$ or amide $-CONH_2$ [9–12]. Currently, the most used hydrophilic polymers in the pharmaceutical industry and pharmaceutical development belong to the group of cellulose derivatives (hydroxypropylmethylcellulose, oxycellulose, etc.), acrylic derivatives, alginates, chitosan, polyoxyethylene, polyvinyl alcohol and thiolated polymers (thiomers) or materials which are able to adhere directly to the cell surface, rather than to mucus [13] such as lectins or bacterial adhesions [10,14–18].

The mucoadhesive process is influenced by many factors that must be taken into consideration during the development of MBFs. The physiological factors of the organism include mucus turnover rate, movements of oral mucosa and affecting the buccal mucosa in disease states. Mucoadhesive bonds are also influenced by three main external factors [9,19–21]: pH (affects the charge of mucoadhesive polymers and mucus molecules [22]); contact time between mucoadhesive film and mucus layer; and application pressure (determines the extent of interpenetration of polymer chains).

Studies of mucoadhesiveness remain one of the critical and essential parameters to be assessed in order to ensure the quality of the prepared MBFs. Despite the intense focus on buccal film-based systems, there are no official standardized methods for their evaluation. Various research groups have employed different methods and experimental conditions for evaluating the formulation, which has limited the possibility of comparison of data between the research groups and the statistical evaluation of the significance of individual factors on the formulation properties [23]. The used methods are usually *in vitro* in nature due to their relative easy implementation and cost-effectiveness, and as such may present an efficient way of selecting candidate delivery systems for further, more expensive *in vivo* testing [10].

In vitro tests for determination of mucoadhesive properties have evolved from simple measurements of force of detachment to more sophisticated setups in recent years [10,24]. The most obvious method used to assess mucoadhesiveness is the determination of the adhesive strength between the attached polymer and the substrate. The adhesive strength at such a bonding interface can be measured as the force required for breaking the adhesive bonds between a model membrane and mucoadhesive form by the application of an external force. The destruction of the adhesive bonds is usually under the application of either shearing, tensile or peeling forces [22,23]. The majority of recent works has been based on tensile-based setups using modified texture analyzers or modified balances [14]. Results of these measurements indicate that mucoadhesive determination could be influenced by variables as especially: the contact force, the contact time, the speed of probe removal from the mucosal surface and the nature of the mucosal surface (artificial, porcine, bovine, rat, rabbit, sheep, dog, primate, hamster mucosa, etc.) [10,24]. A longer contact time and higher probe speed were found to give a greater degree of sensitivity arising from greater reproducibility in results along with higher measurement values. On the contrary, a low probe speed led to good reproducibility for viscoelastic formulations [24]. In terms of the contact force, it was determined that a certain level of force affected the mucoadhesion, beyond which further increases in force had little effect [10,25]. One of the recently employed methods allows the measurement of mucoadhesion under the application of shearing stress. Shear stress measures the force (per unit area) required for sliding the films over the mucus layer in a direction parallel to their plane of contact of adhesion [22,23,25]. The other possibility is focused on the use of a rotating cylinder placed within a dissolution apparatus, wherein the adhesion time of various polymer disks to mucosa was established. Changes in the tested system were visually determined and measured until the entire mucoadhesive dosage form was either disintegrated or detached from the mucosal surface [17,26,27].

Rheological measurements of mucoadhesion can also provide an acceptable representative *in vitro* model of the true *in vivo* behavior of a mucoadhesive polymer. The principle of these methods is based on comparing rheological properties of polymer/mucus blends with the rheological sum of similarly concentrated mono-component mucus and polymer systems. Findings showed that the mucoadhesive polymer/mucus mixtures exhibited synergistic rheological profiles [10,28].

Despite the popularity within the scientific literature of the force of mucoadhesive attachment determination and rheological-based techniques, there are other adhesiveness testing methods which may offer alternative insight into the principle and degree of mucoadhesion under suitable conditions (fluorescent probe method, adhesion weight method, flow channel method, mechanical spectroscopic methods, falling liquid film method, colloidal gold staining method, electrical conductance method, measuring the refractive index by surface plasmon resonance, ellipsometry, atomic force microscopy, etc.) [10,13,22,25,28].

The evaluation of *in vitro* residence time can also be considered as an indirect method of assessing mucoadhesion, though it does not provide specific information about the nature and strength of mucoadhesive bonds between the medical form and the mucosal surface, but only about the time of fixation based on mucoadhesive interactions. High values of the bioadhesion force do not necessarily lead to a long residence time, because bioadhesion phenomenon is based on surface charge density and chain flexibility, whereas the residence time is correlated with the dissolution rate [24]. The *in vitro* residence time is usually practically determined using a modified disintegration apparatus [3,25]. However, this method is not very precise (inaccurate simulation of oral cavity movements/motion), yet it has its merits in terms of simplicity and providing some evidence about the adhesive nature of the film [23].

The problem with methods used for evaluation of mucoadhesive properties is not only the used technique, but also the selection of the test mucoadhesive membrane (mesh, cellophane, filter paper, dialization semipermeable membrane, etc.) covered with mucus or kinds of mucosa [10,29]. The limited availability