Application of Artificial Neural Networks in Prediction of Diclofenac Sodium Release From Drug-Modified Zeolites Physical Mixtures and Antiedematous Activity Assessment

DANINA KRAJIŠNIK,[1] RADICA STEPANOVIĆ-PETROVIĆ,[2] MAJA TOMIĆ,[2] ANA MICOV,[2] SVETLANA IBRIĆ,[1] JELA MILIĆ[1]

[1]Department of Pharmaceutical Technology and Cosmetology, Faculty of Pharmacy, University of Belgrade, Belgrade 11221, Serbia
[2]Department of Pharmacology, Faculty of Pharmacy, University of Belgrade, Belgrade 11221, Serbia

Received 23 October 2013; revised 27 December 2013; accepted 6 January 2014

Published online 4 February 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23869

ABSTRACT: In this study, utilization of artificial neural network (ANN) models [static—multilayer perceptron (MLP) and generalized regression neural networks and dynamic—gamma one-layer network and recurrent one-layer network] for prediction of diclofenac sodium (DS) release from drug-cationic surfactant-modified zeolites physical mixtures comprising different surfactant/drug molar ratio (0.2–2.5) was performed. The inputs for ANNs trainings were surfactant/drug molar ratios, that is, drug loadings in the drug-modified zeolite mixtures, whereas the outputs were percents of drug release in predetermined time points during drug release test (8 h). The obtained results revealed that MLP showed the highest correlation between experimental and predicted drug release. The safety of both natural and cationic surfactant-modified zeolite as a potential excipient was confirmed in an acute toxicity testing during 72 h. DS (1.5, 5, 10, mg/kg, p.o.) as well as DS-modified zeolites mixtures produced a significant dose-dependent reduction of the rat paw edema induced by proinflammatory agent carrageenan. DS antiedematous effect was intensified and prolonged significantly by modified zeolite. These results could suggest the potential improvement in the treatment of inflammation by DS-modified zeolite mixtures. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1085–1094, 2014

Keywords: clinoptilolite; cationic surfactant; adsorption; excipient; diclofenac sodium; neural networks; in silico modeling; dissolution; antiedematous activity; dose-response

INTRODUCTION

A wide range and variety of minerals have been used as excipients in pharmaceutical preparations because they have certain desirable physical and physicochemical properties, such as high adsorption capacity, specific surface area, swelling capacity, and reactivity to acids.[1] More recently, some tectosilicates (zeolites) have emerged as potential materials for biomedical application.[2–4] The discovery that natural zeolites (NZs) (mainly clinoptilolite), could have therapeutic properties or being used as excipients (auxiliary substances) in pharmaceutical preparations has greatly enlarged the field of their applications in many sectors of biology and medicine, opening unexpected horizons for either basic or applied research.[5] Biomedical application of NZs is based on its ion-exchange or adsorption capability and it is strongly connected to the integrity of its structure. The NZs, because of their net negative charge and hydrophilic surface, have no ability to adsorb nonpolar organic molecules as well as inorganic anions. To increase hydrophobicity of the mineral surface providing a high affinity for organic, that is, drug molecules, the chemical modification of zeolitic surface with long chain organic cations has been used.[6] The preadsorbed surfactant works as an anchor for drug adsorption and enables the enhanced coadsorption of drug molecules on the new stable solid–surfactant complexes. This phenomenon, termed surface solubilization, adsolubilization, or coadsorption, is a surface analog of micelle solubilization and may lead to innovative zeolite applications, such as excipients for modified drug delivery.[7–11]

In our previous studies, it was demonstrated that modification of NZ with cationic surfactant hexadecyltrimethylammonium bromide (HB) at different modification levels (10, 20, and 30 mmol/100 g, denoted as ZHB-10, ZHB-20, and ZHB-30) resulted in an increase of the drug (diclofenac sodium) (DS) adsorbed amounts.[12,13] Characterization of the composites before and after the drug adsorption was performed by determination of electrokinetic mobility, FTIR, and thermal analysis and the results indicated interactions between the drug and carriers. *In vitro* drug release investigations revealed that prolonged DS release from the obtained drug-modified zeolite composites during 8 h was achieved and that the maximum DS released amount (up to 30%) was obtained from DS/ZHB-20 composite at surfactant/drug molar ratio (SDMR) (mmol HB/mmol DS ∼ 1.3). Furthermore, it was shown that prolonged drug release was also attained from the corresponding physical mixture of ZHB-20 and DS at similar SDMR (∼1.4) and as well as from physical mixtures with decreasing SDMR (1.0, 0.7, and 0.4), that is, the drug content closer to a therapeutic dose.[13]

Diclofenac, a phenylacetic acid derivative, is a nonsteroidal anti-inflammatory drug (NSAID). It is used mainly as the sodium salt for the relief of pain and inflammation in rheumatic disease (including juvenile idiopathic arthritis) and other musculoskeletal disorders, acute gout, and postoperative pain.[14] The usual oral or rectal dose (DS) is 75–150 mg daily in divided doses. Diclofenac is rapidly absorbed when given as an oral solution, sugar-coated tablets, rectal suppository, or by intramuscular injection. The terminal plasma half-life is about 1–2 h.

Correspondence to: Danina Krajišnik (Telephone: +381-11-3951359; Fax: +381-11-3972840; E-mail: danina@pharmacy.bg.ac.rs)

Journal of Pharmaceutical Sciences, Vol. 103, 1085–1094 (2014)

Diclofenac as NSAID is associated with gastrointestinal disturbances including discomfort, nausea, diarrhea, and occasionally bleeding or ulceration.[15,16] Because of these side effects and its short half-life, it is an ideal candidate for modified release dosage forms where the rate and/or place of release of the active substance(s) is different from that of a conventional-release dosage form administered by the same route. The common goal for the development of any modified release preparation is enhance the drug's therapeutic benefits, minimize side-effects, and improve the overall management of the disease.[17,18]

The purpose of the present study was to investigate the possibility of artificial neural network (ANN) models utilization for prediction of DS release (during 8 h) from drug-modified zeolites physical mixtures on the basis of knowledge of SDMR, that is, drug loading in DS-modified zeolite physical mixtures. An ANN is an intelligent nonlinear mapping system (a mathematical or computational model) built to loosely simulate the functions of the human brain, consisting of many nodes and their connections. Regarding networks architecture, that is, interconnectedness of its elements ANNs can be classified as static and dynamic (recurrent).[19–22]

Furthermore, we examined an acute toxicity to evaluate safety of both natural and cationic surfactant-modified zeolite as a potential excipients, as well as antiedematous effect of DS and DS-modified zeolite physical mixtures in a rat model of localized inflammation.

MATERIALS AND METHODS

Materials

A sample of NZ-rich tuff from Zlatokop deposit (Vranje, Serbia) (ZVB) was used as the starting material for the preparation of the composite. On the basis of qualitative X-ray powder diffraction analysis, the clinoptilolite content was approximately 80% with trace amounts of quartz, pyrite, and feldspar. The complete chemical characterization of the starting zeolitic tuff was given previously.[10,23,24] Cationic surfactant HB (Sigma–Aldrich, St. Louis, Missouri) was used for the preparation of ZHB-20. Potassium dihydrogen phosphate and sodium hydroxide (Lach-Ner, Brno, Chech Republic) were used for buffer preparation, whereas DS (of pharmacopoeial quality) was supplied directly from the pharmaceutical industry (Galenika, a.d., Belgrade, Serbia) and used without further purification. Double distilled water was used throughout the experiments.

Methods

Preparation of the Modified Zeolite–Drug Composites and Modified Zeolite–Drug Physical Mixtures

For that purpose, 10% aqueous (w/w) suspension of ZVB was treated with HB equivalent to 200% of ZVB external cation-exchange capacity—*ECEC*. The cation-exchange capacity of the starting material was 146 mmol M^+/100 g measured by the ammonium chloride method, whereas its *ECEC* was 10 mmol M^+/100 g.[23] Details of the zeolite modification procedure are given elsewhere.[10] In brief, the adsorption reaction was carried out using a laboratory mixer at 5000 rpm and 50°C with an activation time of 15 min. After mixing, the suspension was filtered using ashless filter paper for achieving extremely fine precipitates. The filtrates were further centrifuged at 1500 × g for 20 min and the supernatant was used for HPLC determination of the surfactant concentration. The adsorbed surfactant amount was calculated as the difference between the initial and surfactant concentration in the supernatant after the modification procedure. The obtained composites were washed with distilled water and dried in an oven during 2 h at 60°C.

Modified zeolite–drug physical mixtures containing DS and ZHB-20 in different (mmol HB/mmol DS) ratios (from 0.2 to 2.5) were prepared by geometric dilution using a pestle and mortar. The mixtures were ground for 15 min to assure adequate homogenization.

Characterization of the Modified Zeolite–Drug Composites and Modified Zeolite–Drug Physical Mixtures

Drug Release Determination. The pharmaceutical performance of modified zeolite–drug physical mixtures was evaluated by *in vitro* dissolution experiments. The flat-faced punches with a diameter of 9 mm were used to compress the tested physical mixtures into 200 mg comprimates using an eccentric compressing machine (EKO Korsch, Berlin, Germany).

Details on determination of DS and conditions for dissolution study were described in previous papers.[10,25]

Computational Methods. Artificial neural network analysis was performed with commercial software, Synapse (Version 1.3.5; Peltarion, Stockholm, Sweden). Inputs were: (1) SDMR and (2) drug loading in the drug/modified zeolite (mg/g) mixture. Outputs were percents of drug release in 12 predetermined time points during drug release test (after 10, 20, 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min).

Data were classified into three groups, that is, a training data set, a validation data set, and a test data set. The validation samples were 15% of the training samples and were selected randomly by the software. The network was trained using a batch training procedure where all samples in the training data set were packed together and passed through the network as a single signal. All outputs in the network were trained simultaneously. During the training process, the network performance was validated by a cross-validation method using samples from the validation data set at every five training iteration. This validation was assigned to test the adaptive system on the data that had not been used for training to benchmark the system performance. After the training process, prediction ability of the developed network was examined by external validation with the unseen samples of the test data set.

Drug release profiles were modeled using both static and dynamic neural networks. Multilayer perceptron (MLP) and generalized regression neural networks (GRNN) were used as static networks, whereas gamma one-layer network (GOL) and recurrent one-layer network (ROL) were use as dynamic networks. Topologies of all networks used in this study are presented in Figure 1.

Mutilayer perceptron layout is given in Figure 1a. From data source 1, signals go to the first weight layer (with two inputs and seven outputs) and first function layer (with seven inputs and seven outputs). Function layer applies Tanh sigmoid function to input data and the number of outputs was optimized using Monte Carlo simulations tool in the training mode of the software. From the first functional layer, signal goes to the second weight layer (with seven inputs and 12 outputs) and then to the second function layer (12 inputs and outputs for 12 sampling points in dissolution time profile). Finally, Delta