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# Preparation of agar nanospheres: Comparison of response surface and artificial neural network modeling by a genetic algorithm approach

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ABSTRACT

Multivariate nature of drug loaded nanospheres manufacturing in term of multiplicity of involved factors makes it a time consuming and expensive process. In this study genetic algorithm (GA) and artificial neural network (ANN), two tools inspired by natural process, were employed to optimize and simulate the manufacturing process of agar nanospheres. The efficiency of GA was evaluated against the response surface methodology (RSM). The studied responses included particle size, poly dispersity index, zeta potential, drug loading and release efficiency. GA predicted greater extremum values for response factors compared to RSM. However, real values showed some deviations from predicted data. Appropriate agreement was found between ANN model predicted and real values for all five response factors with high correlation coefficients. GA was more successful than RSM in optimization and along with ANN were efficient tools in optimizing and modeling the fabrication process of drug loaded in agar nanospheres.


## 1. Introduction

Incorporating the drugs into the matrix of polymers is one strategy to slow the release rate of the drug and naturally occurring polymers are preferred compared to synthetic ones. Among various natural polymers available, agar and agarose (a fractions of agar) have been used to prepare sustained release matrices including micro and nanoparticles by several researchers (Bera, Sarwa, & Mazumder, 2013; Lee, Park, Khan, & Lim, 2011; Linghui et al., 2011; Wang & Wu, 1997, 1998).

Agar shows some interesting physical properties. It is not soluble in cold water but is soluble in boiling water. Its solution forms thermally reversible hydrogels while being cooled down below its gelation temperature (31–36 °C). The reversible gel-to-sol transition for the agarose hydrogel does not occur below the melting point (65–85 °C) (Selby & Wynne, 1973). This exceptional behavior of agar has been exploited to fabricate nanosphere in an interesting process which includes the conversion of a water in oil emulsion to a solid in oil suspension the solid phase of which entraps the active drug.

Due to the complex nature of the development of pharmaceutical formulations, some computer-based optimization techniques have been proposed in the recent years. Among them response surface methodology (RSM) is the most widely used (Douroumis, Hadjileontiadis, & Fahr, 2006). However, the optimization capability and prediction efficiency of RSM is often restricted to low levels since it is based on simple first or second-order polynomial equations (Sun, Peng, Chen, & Shukla, 2003; Takayama, Fujikawa, & Nagai, 1999; Takayama, Fujikawa, Obata, & Morishita, 2003), and therefore, it sometimes results in poor estimation of optimal formulations. In the field of optimization and prediction several techniques such as genetic algorithms (GA), artificial neuron networks (ANN), or their combinations have been proposed.

Genetic algorithm is a heuristic optimization technique inspired by natural evolution. It has been successfully applied for a wide range of real-world problems of significant complexity (McCall, 2005). Tao and Zhang (2005) have proposed a genetic algorithm–based area coverage approach for controlled drug delivery using micro robots. Their proposed GA approach had advantages of reducing computational costs. In another study Varshosaz, Moazen, and Fathi (2012) used neural network coupled with GA to predict the release behavior of carvedilol loaded nanoparticles.

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Artificial neural network (ANN) is a learning system that simulates the neurological processing ability of the human brain and it has been successfully applied to address various pharmaceutical research problems (Achanta, Kowalski, & Rhodes, 1995), achieving more accurate predictions than those predicted by polynomial equations (Takayama et al., 1999). Ghaffari et al. (2006) applied GA to train artificial neural networks in modeling the release profile of theophylline for bimodal drug delivery. They applied different training algorithms including incremental back propagation (IBP), batch back propagation (BBP), quick propagation (QP), quasi-Newton (Levenberg–Marquardt, LM) and genetic algorithm (GA) to train ANN. The precision of predictive ability was measured for each training algorithm and their performances were in the order of IBP & BBP ($R^2$ = 0.9815) > LM ($R^2$ = 0.9517) > QP ($R^2$ = 0.9344) > GA ($R^2$ = 0.8329).

Bupropion is an antidepressant drug of the aminoketone class which inhibits the reuptake of dopamine and norepinephrine (Brunton, 2011; Laizure, DeVane, Stewart, Dommisse, & Lai, 1985; Stahl et al., 2004). Bupropion is one of the short-acting antidepressants that have been prepared in slow-release preparations to allow less frequent dosing (Brunton, 2011). The aim of this study was to optimize the bupropione HCl loaded agar nanospheres characteristics exploiting genetic algorithm and response surface methodology and modeling of the process using ANN.

## 2. Materials and methods

### 2.1. Chemicals

Agar was obtained from Narico (Germany), liquid paraffin from Golnoosh Company (Iran), hydroxypropyl beta cyclodextrin (HPβCD) was purchased from Sigma (US), Bupropion HCl from Dipharma (Italy), calcium chloride and methanol HPLC grade from Merck Chemical Company (Germany). All other reagents were of analytical grades.

### 2.2. Preparation of nanospheres

Agar (100, 150, and 200 mg) was added to 10 ml of water. The resultant suspension was heated up to boiling temperature to dissolve the agar and then cooled down to 45 °C keeping the vessel covered meanwhile to prevent the loss of water. Calcium chloride (as the cross linker of agar polysaccharide), HPβCD (as the absorption enhancer of the drug), and Bupropione HCl (as the active ingredient) previously dissolved in appropriate amounts of water (respective amounts are shown in Table 1) were added at the same temperature and were mixed for 3 min. The resultant solution was added under homogenization to 40 ml of liquid paraffin previously warmed up to 40 °C. After homogenizing for 2 min the suspension was cooled down below 20 °C by immersing the vessel in ice water bath. Centrifugation at 8000 rpm for 5 min was performed to settle the nanospheres. The sediment was washed three times by re-dispersing in 5 ml of methylene chloride. The final sediment was dispersed in a sufficient amount of 5% mannitol solution as a cryoprotectant and subsequently was freeze-dried.

### 2.3. Particle size and zeta potential measurement

Particle size and potential measurement was performed by Malvern zeta sizer (Model 3000 HS, UK).

### 2.4. In vitro drug release studies

Nanospheres equivalent to 2.5 mg of Bupropione HCl was dispersed in 3 ml of phosphate buffer, decanted in dialysis bag and placed in 200 ml of 37 °C phosphate buffer solution (pH 7.2). At predetermined time intervals 3 ml of release medium was withdrawn and analyzed spectrophotometrically at 298 nm to determine the released amount of Bupropione HCl. The withdrawn samples were replaced by 3 ml of 37 °C fresh buffer.

Release efficiency at 300 min ($RE_{300}$) of release test was calculated according to the following equation for each formulation:

$$RE_{300}\% = \frac{\int_0^{300} y \times dt}{y_{100} \times t} \times 100 \quad (1)$$

where $y$ is the released percent of the drug at time $t$. Lower RE values mean slower release of the drug in the studied period.

### 2.5. Drug loading efficiency determination

Accurately weighed 40 mg of nanoparticles was dispersed in 20 ml of phosphate buffer (pH 7.2), shacked for 24 h and assayed after filtration through 0.22 μm syringe filter by high pressure liquid chromatographically according to Loboz, Gross, Ray, and McLachlan (2005) with some modifications.

Drug loading efficiency (LE) was calculated according to the following equation:

$$LE = \frac{L_A}{L_T} \times 100 \quad (2)$$

where $L_A$ and $L_T$ are actual and theoretical loaded Bupropion HCl, respectively.

### 2.6. Optimization

In this study optimization was performed using RSM as well as GA and the results were compared.

#### 2.6.1. Response surface methodology (RSM)

In this study Design-Expert software (version 7.0.0, Stat-Easc, Inc., Minneapolis, MN, USA) was used to develop a D-optimal RSM design based on the independent factors calcium chloride percent (A), homogenization speed (B), agar percent (C), and HPβCD percent (D). Optimization for response factors included minimizing the particle size, $RE_{300}$, and pdi while, maximizing the absolute value of zeta potential and the loading efficiency. Making use of this design, the best models among the linear, two-factor interaction model and quadratic model were chosen based on the analysis of variance (ANOVA) through Fisher's test. The $P$-value less than 0.05 was considered to be statistically significant.

Solution provided by the software with the greatest desirability was chosen as the optimum condition and after executing the experiment based on the suggested values for the independent factors the real responses were compared with the predicted ones and error percent was calculated to evaluate the predictive ability of the models.

#### 2.6.2. Genetic algorithm (GA)

GA is a heuristic solution-search or optimization technique, originally motivated by the Darwinian principle of evolution through genetic selection. Genetic algorithms were first proposed by John Holland (Holland, 1975) as a mean to find good solutions to problems that were computationally intractable (Ding, Li, Su, Yu, & Jin, 2013). Starting with a randomly generated population of chromosomes, a GA carries out a process of fitness-based selection and recombination to produce a successor population, the next generation. During recombination, parent chromosomes are selected and their genetic material is recombined to produce child chromosomes. These then pass into the successor population. As this process is iterated, a sequence of successive generations evolves