PREPARATION OF ACETAZOLAMIDE DRUG DELIVERY SYSTEMS USING A SUPERCRITICAL ANTISOLVENT PROCESS

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The possibility of preparing ophthalmic drug delivery systems using supercritical antisolvent technology was evaluated. Eudragit RS 100 and RL 100 were used as drug carriers and acetazolamide was the model drug processed. SAS experiments were performed at different operational conditions, and different formulations of polymer/drug were prepared. Experiments were performed using a liquid solution of the polymer + solute in acetone. The particles obtained were analysed by SEM and the particle size was determined.

INTRODUCTION

Glaucoma is a serious eye disease that can lead to irreversible blindness. Glaucoma is a group of ophthalmic disorders characterized by an increase in intraocular pressure, which results in damage to the optic nerve and visual field disturbances. Agents used to treat glaucoma are designed to decrease intraocular pressure. Nevertheless, these drugs present severe side-effects therefore, there is an urgent need to develop new drug delivery systems. Various classes of drugs used in the treatment of glaucoma include, among others, carbonic anydrase inhibitors (CAIs). Acetazolamide (*N*-5-sulfamoyl-1,3,4-thiadiazol-2-yl)-acetamide (Figure 1) is a CAI and has been an integral part of antiglaucoma treatment for more than 40 years.[1]

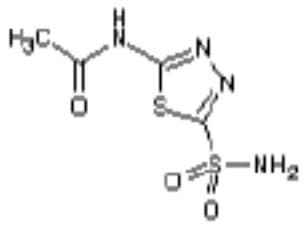

Figure 1: Chemical structure Acetazolamide

Earlier studies have reported the preparation of drug-loaded nanospheres of Eugragit® RS 100 and RL 100 for controlled delivery of ophthalmic drugs, namely acetazolamide.[2]

Modern sustained release dosage forms require excipients that can ensure a release rate of the active drug which is reproducible in a narrow range. Eudragit® polymers fulfil this requirements to a very high extend and enable research and development to create tailor-made solutions.[3]

The Eudragit® grades for sustained release formulations are based on copolymers of acrylate and methacrylates with quaternary ammonium groups as functional groups as well as ethylacrylate methylmethacrylate copolymers with a neutral ester group. Eudragit® RS and RL are water-insoluble nevertheless they are both swellable, i.e., permeable to water representing interesting materials for the dispersion of drugs.[4] This permeability is due to the quaternarium ammonium groups present in their structure (Figure 2). The Eudragit® RL-types are highly permeable, the Eudragit® RS-types are poorly permeable; therefore the release profiles can be determined by varying mixing ratios.

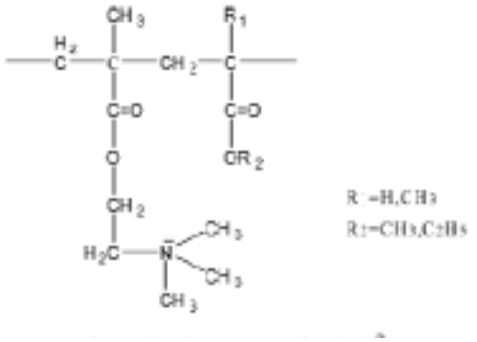

Figure 2: Chemical structure of Eudragit®

Nanoparticle coating and encapsulation of silica particles with Eudragit® has been successfully described by Wang *et al*.[5] Furthermore, the preparation of Eudragit® microspheres containing acetazolamide using the solvent evaporation method was appraised by Haznedar *et al*.[2] In this study, the possibility of preparing ophthalmic drug delivery systems using supercritical antisolvent (SAS) technology was evaluated. Eudragit® RS 100 and RL 100 were used as drug carriers and acetazolamide was the drug processed. SAS experiments were performed at different operational conditions and different compositions of polymer/drug were prepared.

MATERIALS AND METHODS

Materials

Eudragit RL 100 and RS 100 were purchased from Degussa. Acetazolamide, (*N*-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide, CAS [59-66-5] (99% purity), was purchased from Sigma-Aldrich. Acetone CAS [67-64-1] (99.8% purity) was purchased from Prolabo (France). CO2 (99.5%, industrial grade) was obtained from Air Liquide (France). All chemicals were used without any further purification.

Experimental Apparatus and procedure

The experimental work was performed at a laboratory scale. A SAS apparatus, similar to the one used, is schematically presented in figure 3. Experiments were performed in a similar manner to that described by Vega *et al*.[6] The apparatus works in a semi-continuous co-current mode and it consists in a precipitator in which the antisolvent and the liquid solution are separately fed to the top of the chamber and are continuously discharged from the bottom.

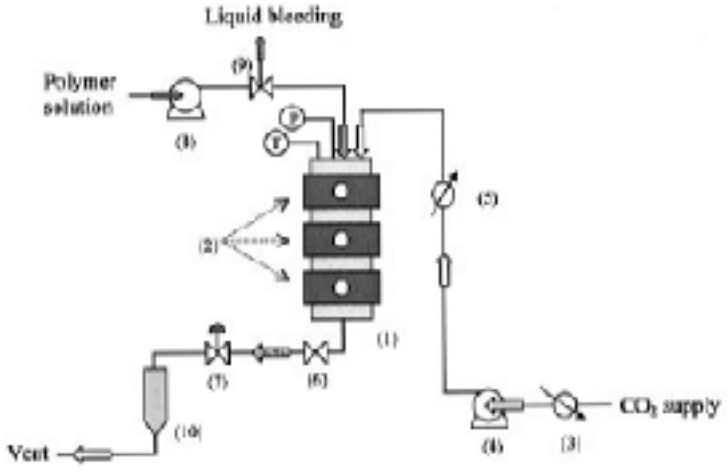

Figure 3: Experimental apparatus: (1) high-pressure vessel; (2) sapphire windows; (3) CO2 cooling bath; (4) CO2 pump; (5) CO2 heating bath; (6) valve; (7) micrometering valve; (8) liquid solution pump; (9) three-ways valve; (10) separator.

The spray chamber (1) consists of a high-pressure vessel (Autoclave Engineers), 5 cm i.d. x 25 cm long, rated to 320 bar at 150°C, with sapphire windows at three different levels (2) allowing a visual observation of both the spray and the precipitation. At the bottom of the vessel, the precipitated polymer is collected onto a membrane filter placed on top of a

stainless steel filter of 2 μm porosity. The CO_2 was cooled by flowing through a cold water + ethanol bath (3) and then was pumped at constant flow by using a reciprocating LEWA (EK3) pump (4). Prior to entering the precipitation chamber, it flowed through a second water bath (5) to be preheated at the vessel temperature. The pressure inside the vessel was controlled downstream with an Autoclave Engineers micrometering valve (7), and the temperature was controlled by heating jackets (Watlow). Acetone solutions of the solutes were sprayed into the precipitation chamber by means of a reciprocating dual-piston minipump (Milton Roy LDC). Once the temperature of the vessel had attained the desired value, the CO_2 was pumped to the high-pressure vessel via a 1/8" in tube, keeping the valve (6) closed, until the desired pressure was reached. Then, the valve (6) was opened and the system was allowed to equilibrate, maintaining constant the CO_2 flow. The solution was introduced into the precipitation chamber through a spray-type nozzle of 100 μm. After leaving the precipitator, the CO_2/acetone solution was depressurised across the metering valve and separated in a homemade cyclonic separator (10). Once the solution was sprayed, fresh CO_2 passed through the chamber in order to dry the precipitated. After approximately 40 min of purging with CO_2, the vessel was slowly depressurized, at the experimental temperature. Tubing and valves after the vessel were heated to prevent freezing due to CO_2 expansion, and they were flushed with acetone between each experiment.

Microspheres Characterization

Scanning electron microscopy (SEM)

The morphology of polymer samples was analysed and imaged by scanning electron microscopy (SEM, Leica 5440) after sputter coating with gold-palladium to a thickness of approximately 90 Å.

Particle size and size distribution

The particle size and size distribution of the prepared microparticles were measured by Laser diffraction spectrometry (Coulter LS 130, Coulter Electronics). The dried powder samples were suspended in deionised water with a surfactant solution (Coulter Dispersant, Coulter) and sonicated for 1 minute with an ultra-sound probe (500 W, Vibra Cell, Sonics & Materials, Inc.) before measurement. The obtained homogeneous suspension was determined for the

volume mean diameter, size distribution and polydispersity. Each sample was analysed 3 times.

RESULTS AND DISCUSSION

Diferent experiments were performed in order to determine the best operational conditions for the precipitation of the solution. A summary of the experiments carried out is listed in the following table. The first experiment was performed based on the results from Wang *et al*.[5] The effect of pressure, temperature and CO2 flow were evaluated and a compromise between the particle size, particle size distribution and yield of the process was established. The best operating conditions to perform the subsequent experiments are 83 bar and 37 ºC with a CO2 flow rate of c.a. 15 mL/min. All experiments were carried out with a organic solvent flow of 1 mL/min.

# experiment	Polymer	Drug	Pressure (bar)	Temperature (ºC)	CO_2 flow (ml/min)	Yield (%)	Particle size (μm)
1	Eudragit RL 100	Acetazolamide	82	32	15.1	39	20.07
2	Eudragit RL 100	Acetazolamide	100	32	15.8	57.2	16.28
3	Eudragit RL 100	Acetazolamide	100	37	16	50.1	15.3
4	Eudragit RL 100	Acetazolamide	100	37	29.22	33	18.47
5	Eudragit RL 100	Acetazolamide	83	37	15.18	77	17.85
6	Eudragit RS 100	Acetazolamide	83	37	15.40	63	23.88
7	RL 100 + RS 100 (50:50)	Acetazolamide	83	37	15.27	66	21.32
8	RL 100 + RS 100 (70:30)	Acetazolamide	83	37	15.4	70.7	19.76
9	RL 100 + RS 100 (30:70)	Acetazolamide	83	37	15.8	74.8	17.04

Additionally, solutions with different polymer ratios were coprecipitated with acetazolamide and a 3:1 polymer:drug concentration.

The morphology of the particles obtained is similar for all experiments and as an example a SEM image of the particles precipitated is shown in figure 4.

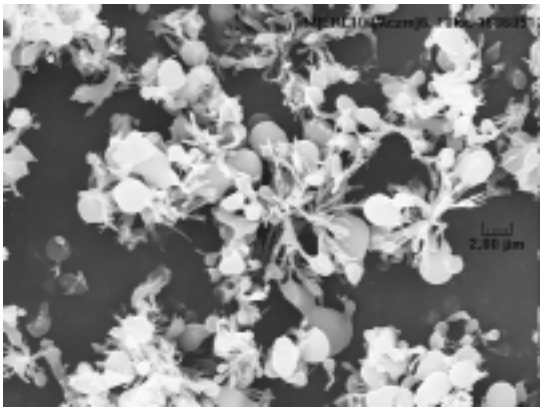

Figure 4: SEM image of particles precipitated by SAS, experiment 5

CONCLUSIONS

The preparation of acetazolamide composite particles for ophthalmic drug delivery was successfully performed. Different experiments were carried out and the best operational conditions for the precipitation of these systems are 83 bar, 37 ºC with 1 mL/min liquid flow and 15 mL/min CO2 flow. Future work, involves the study of the release profiles of the systems prepared and the correlation with the composition of the blends prepared.

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