IN VITRO EVALUATION OF DIFFUSION AND RHEOLOGICAL PROFILES FOR DEXAMETHASONE INCLUSION COMPLEXES WITH β-CYCLODEXTRIN OR HYDROXYPROPYL β-CYCLODEXTRIN

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Abstract

Dexamethasone (Dmt), is very potent glucocorticoid, and inclusion complexes formed with cyclodextrins (Cd), enhance skin penetration. Five gels were prepared representing combinations of the drug with lyophilized or simple physical mixtures of β-cyclodextrin (Bcd), or hydroxypropyl β- cyclodextrin (HBcd), in the 1:1 molar ratio. The paper presents *in vitro* measured values of these forms by using a vertical diffusion cells system and a rotational rheometer. The experimental conditions represented an adaption of the USP Topical/Transdermal Ad Hoc Advisory Panel stimuli for revision process.

The aim of this study was to evaluate the improving of skin penetration by Dmt in different mixtures with two Cd, by using the drug release and rheological profiles.

Diffusion coefficients of Dmt inclusion complexes with Cd significantly increased compared with the single use of Dmt. The best released rate of Dmt was obtained for the lyophilized inclusion complex with Bcd. A significant diffusivity increase was noticed for the physical mixtures. Data fitted five kinetic models approximating the diffusion profiles. The Korsmeyer–Peppas model gave the best correlation between measured and interpolated data.

All the rheological investigated gels presented shear-thinning and thixotropic behavior. A nonlinear model fitted the rheological data. The obtained profiles suggest better performances of Dmt inclusion complexes for topical applications.

Rezumat

Dexametazona (Dmt), este un glucocorticoid foarte potent, iar complexul de incluziune format cu ciclodextrinele (Cd), accentuează penetrarea prin piele. Au fost

formulate cinci combinații posibile ale substanței medicamentoase liofilizate sau în amestecuri mecanice simple cu β-ciclodextrină (Bcd), hidroxipropil β-ciclodextrină (HBcd) , în raport molar 1:1. În articol sunt prezentate valorile măsurate ale acestor formulări, utilizând un sistem cu celule de difuzie verticale si un reometru rotational.

Scopul acestui studiu a fost acela de a evalua îmbunătățirea penetrării pielii de către Dmt în diferite amestecuri cu două Cd, folosind profilele de cedare a medicamentului și cele reologice.

Coeficienții de difuzie ai complecşilor de incluziune Dmt cu Cd au crescut semnificativ în comparație cu utilizarea singulară a Dmt. Cea mai ridicată rată de cedare a Dmt a prezentat-o complexul de incluziune cu Bcd realizat prin liofilizare. O creştere semnificativă a puterii de difuzie a fost observată și la amestecurile fizice. Folosind datele experimentale, au fost realizate cinci modele care aproximează profilele de difuzie. Modelul Korsmeyer–Peppas a prezentat cea mai bună corelație între datele măsurate și cele interpolate.

Toate gelurile investigate reologic au arătat subțiere prin forfecare și comportament tixotropic. Pentru modelarea datelor reologice s-a folosit un model neliniar. Profilurile obținute sugerează performanțe mult mai bune pentru complecşii de incluziune ai Dmt în aplicațiile topice.

Keywords: dexamethasone, cyclodextrins, vertical diffusion cell system, topical drug product

Introduction

Most inflammatory diseases occur locally and near the surface of the body, so topical application of drug on the affected site can offer the advantage of direct delivery at the disease site and producing its local effect. Dexamethasone, Dmt, a synthetic glucocorticoid which is about 80 times more potent than the natural one, cortisol, is widely prescribed as immunosuppressive to treat a broad range of autoimmune and inflammatory disorders and in order to prevent graft rejection following bone marrow or organ transplantation. However, the barrier properties of intact skin limit the Dmt permeability.

On the last decade there has been an increase of interest on semisolid dosage forms [1] for drugs that are topically delivered by the skin. The literature survey reveals that its solubility can be enhanced by using cyclodextrins, Cd [2]. They modify transdermal drug penetration by complexation and drug release acceleration by enhancing the proportion of diffusible substance. The inclusion complex was prepared as a semisolid dosage form. *In vitro* release tests for this product employed the vertical diffusion cell, VDC, system. The VDC system is simple to operate and yields reliable and reproducible results. The implemented experimental protocol aimed to test product quality by evaluation of the biopharmaceutical profiles in accordance with the recommendations from

The Topical/Transdermal Ad Hoc Advisory Panel for the USP Performance Tests of Topical and Transdermal Dosage Forms [3, 7]. The drug delivery was investigated by the dissolution profile evaluation. The rheological behavior may affect Dmt application to treatment site and consistency of treatment and thus the delivered dose [4, 6].

The present study presents the evaluation of the dissolution and rheological profiles of Dmt from formulas of aqueous gels with two different Cd. The selected cyclodextrins were: β-cyclodextrin, Bcd - the poorest water soluble, and hydroxypropyl β-cyclodextrin, HBcd - the most powerful water soluble. The mixtures were prepared by two methods: lyophilization or simple physical mixtures in the 1:1 molar ratio. The reference structure was Dmt. All the hydrogels were prepared with the same excipients.

Materials and Methods

The following products were purchased from Sigma Aldrich: dexamethasone, β-cyclodextrin, hydroxypropyl β-cyclodextrin; isopropanol, ethanol absolute, glycerin, and carbomer 940. The purified water was generated by a SGW Ultraclear UV PlusTM system. All the used chemicals are in according to the stipulations of Romanian Pharmacopoeia the Xth edition, United States Pharmacopeia 27 and European Pharmacopoeia 5th edition [5, 7].

The appropriate quantity of the gelling agent Carbopol 940 was added to purified water and mixed for about 30 min. Appropriate and preweighed amounts of wetting agents: glycerin, isopropanol, and ethanol absolute, and drug were then added to the mixture and agitated for additional 20 min. The dispersion was then allowed to hydrate and swell for 60 min. The concentrations (weight fractions) of different components for 0.1% Dmt formulations are presented in Table 1. The selection of the excipient concentrations was carried out by studying their influences on the aspect, transparency and texture of the gels and on sensory preferences. Throughout the study, the abbreviation Dmt denotes the gel containing only Dmt, while the abbreviations Dmt+Bcd and Dmt+HBcd are for gels of Dmt inclusion complexes with β-Cd or hydroxypropil β-Cd, respectively (the complexes were obtained by the lyophilization process and a 1:1 molar ratio is between Dmt and Cd). Dmt-Bcd and Dmt-HBcd were obtained as simple physical mixtures in 1:1 molar ratio between Dmt and Cd (Table I).

Table I

Formulations of 0.1 g % dexamethasone gels

Ingredients	Formulations [g]				
	Dmt	**Dmt+Bcd**	**Dmt-Bcd**	**Dmt-HBcd**	**Dmt+HBcd**
Dexamethasone	0.1	0.1	0.1	0.1	0.1
β-cyclodextrin	-	2.89	2.89	-	-
Hydroxypropyl β-cyclodextrin	-	-	-	3.71	3.71
Carbomer 940	1	1	1	1	1
Glycerin	10	10	10	10	10
Ethanol absolute	20	20	20	20	20
Isopropanol	5	5	5	5	5
Purified water	Up to 100				

Microette Plus^{TM} system was used to study of transfer kinetics. The system has 6 vertical diffusion cells, VDC -10mL effective volume, for *in vitro* Dmt diffusion testing of the transfer rate across a membrane. The VDC diameter is 15mm, corresponding to a surface diffusion of 1.77 cm^2.

A mixture of ethanol-purified water (30:70, v/v) was degassed by using a 0.45 membrane for filtration. The receptor compartments of VDC were filled up with this solution. The resident air bubbles were eliminated by using a magnetic stirrer at 1000 rpm and 30 min for hydro-alcoholic solution. The temperature of diffusion medium, simulating skin temperature, was set up at 32ºC ± 0.2º C, and thermostatically controlled by using a Lauda Ecoline Star edition E100 / 090 thermostat. About 300 mg of semisolid topical dosage forms were put in each donor compartment of VDC, and the stirring rate was set at 400 rpm.

The samples were withdrawn at the following predetermined intervals 30, 60, 90, 120, 150, and 180 min, and replaced by equal volumes of fresh fluid. The samples withdrawn were spectrophotometrically estimated at 240.1 nm against the respective blank.

After application of the topical formulation, the inclusion complexes have to be released from the gel, a vehicle, in the stratum corneum simulated *in vitro* by a membrane, and to diffuse in the receptor compartment. Diffusion is a kinetic process taking place along the concentration gradient. Time and space evolutions of the process can be described by Fick laws: first law for steady state or second law for transient one. On the other hand, diffusion depends on the physicochemical properties of compound diffusion and diffusional medium, viscosity, temperature and pressure. The dependency is given by the Einstein diffusion equation [5]. Therefore, outside the diffusion profile evaluation, the rheological profile has to be assessed.

Viscosity and share stress on ascending and descending route *versus* the share rate of 3mL volume of gel from each formulation was investigated by the Rheometer type RC1, RheoTec GmbH, Germany. The rheometer having a CC14 coaxial cylinder can measure viscosity values up to 10^3Pa.s, and generates share rates from $0.9s^{-1}$ to 10^3s^{-1}.

Results and Discussion

The quantitative determination of Dmt from collected samples of VDC was performed using a Jasco UV-Vis V-530 spectrophotometer in the range 200-450nm. Prior to analysis, the samples were diluted 1:9 with the receptor media, and nine samples having 0.05-10μg/mL were generated and averaged for establishing the optimal wavelength of absorbance. The maximum absorbance was obtained at 240.1nm (Figure 1, r^2>0.9999).

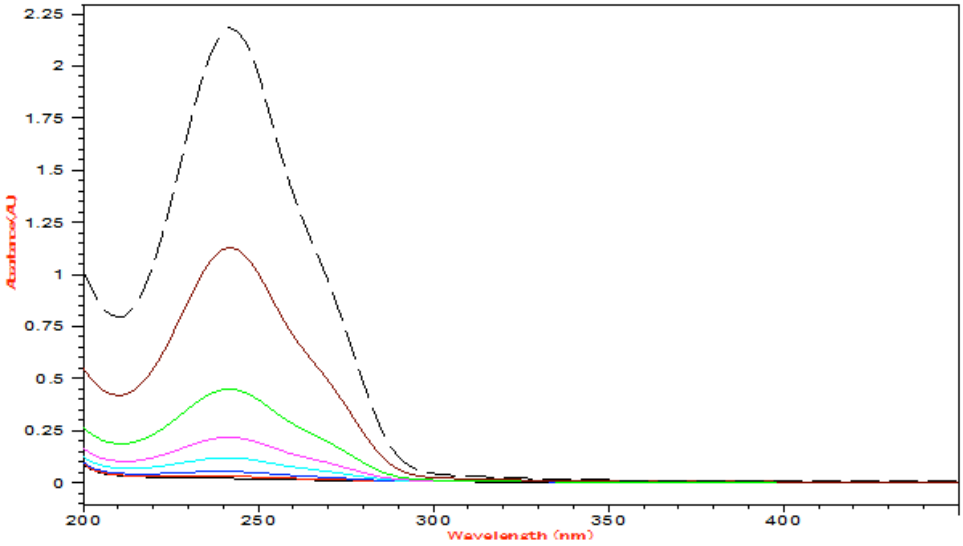


Figure 1

Influence of the Dmt concentration *versus* the gel transparency

Experiments were performed in triplicate, the measured data during the dissolution tests were averaged, and final values were plotted showing diffusion profiles of Dmt and its inclusion complexes (Figure 2).

Dmt releasing can be modeled by using some kinetic models [8]. In this paper, the selected models simulate the cases when the drug release rates could be: independent on concentration, as zero order kinetic (1), dependent on concentration, as first order kinetic (2); dependent on changing of surface area and diameter particles, as Hixson-Crowell curve –

(3) or showing a Fickian diffusion, as Higuchi curve (4). A more general model was proposed by Korsmeyer–Peppas (5). In order to realize a linear regression analysis of the measured data for dissolution curve, the initial equations were modified by taking in consideration of linear time dependence, as follows:

$$C = K_0 t + k_1 \quad (1)$$

$$\log(C) = K_e t + k_2, \quad (2)$$

$$Q^3 = K_{HC} t + k_3, \quad (3)$$

$$Q^2 = (2ADC_d)^2 t = K_H t + k_4, \quad (4)$$

$$\left(\frac{Q_t}{Q_\infty}\right)^n = K_{KP} t + k_5 \quad (5)$$

where C is the Dmt concentration, Q is the amount of Dmt released up to time t, and K_0, K_e, K_H, K_{HC}, K_{KP}, k_1-k_5 are constants.

The measured values which fit the five kinetic models are summarized in Table II.

Table II

Parameters of linear interpolation for kinetic models

Kinetic model		**Dmt**	**Dmt-Bcd**	**Dmt+Bcd**	**Dmt-HBcd**	**Dmt+HBcd**
Zero order	K_0	0.0246	0.0007	0.0076	0.0093	0.0093
	k_1	0.9439	3.9171	3.6917	3.7204	3.7204
	r^2	0.9531	0.9869	0.9638	0.9813	0.9895
First order	K_e	0.7551	0.5827	0.6616	0.8861	1.03
	k_2	-18.3669	35.0567	43.0399	28.97	36.2327
	r^2	0.9888	0.9733	0.9928	0.9963	0.9977
Hixson-Crowell	K_{HC}	0.0927E5	0.1366E5	0.227E5	0.036E6	0.0598E6
	k_3	-4.084E5	-2.651E5	-5.29E5	-1.143E6	-1.902E6
	r^2	0.9039	0.9925	0.9819	0.967	0.9626
Higuchi	K_H	84.8	98.9645	136.468	195.9	271.1
	k_4	-3303	-80.9168	-316.92	-3249.2	-4417
	r^2	0.9911	0.9911	0.9974	0.9938	0.9916
Korsmeyer–Peppas n=0.47	K_{KP}	0.2854	0.194	0.2116	0.281	0.3151
	k_5	-4.6771	17.826	21.1049	16.4545	19.5526
	r^2	0.9917	0.9957	0.9981	0.9923	0.9945

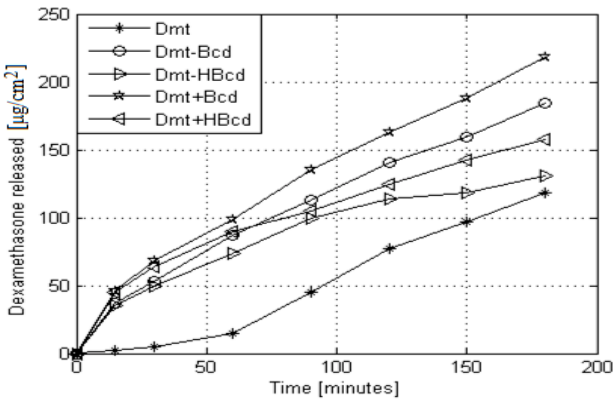
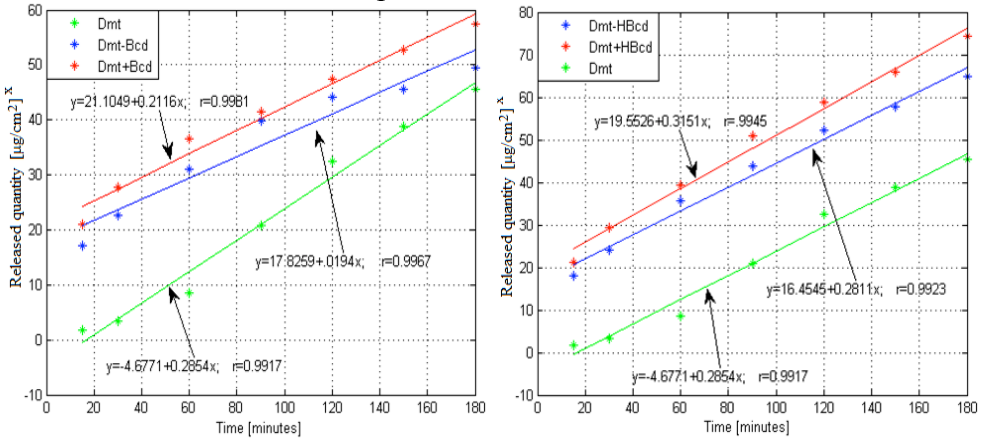


Figure 2

Dissolution profiles for the release Dexamethasone

As a general observation, Korsmeyer–Peppas kinetic model having n=0.47 is the representative model for Dmt release from the pharmaceutical formulas based on the gelling agent Carbomer 940. It offers the best approximation of all the measured values, and Higuchi kinetic model, n=0.5, is quite close to it. The final results of linear interpolation by using Korsmeyer–Peppas kinetic model are shown in Figure 3. So, the Dmt release is a Fickian diffusion process.



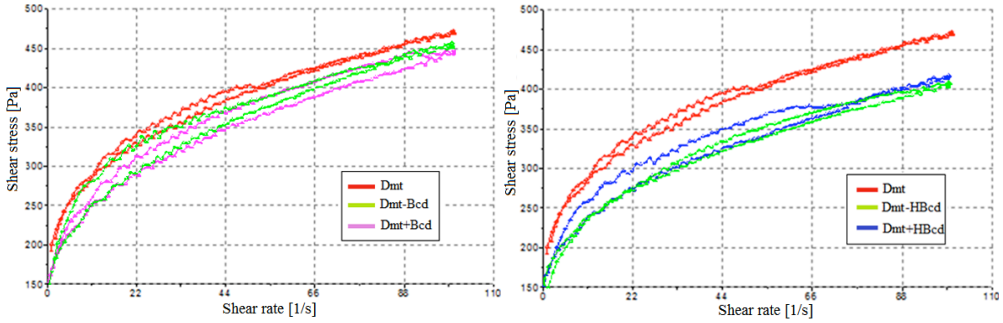
(a) (b)

Figure 3

Korsmeyer -Peppas models for Dmt with Bcd mixtures (a) and Dmt with HBcd mixtures (b)

Modeling by Hixson-Crowell is not realistic enough because it shows a weak dependence of drug release on changing of surface area and particle diameters. Data analysis used to fit zero or first order kinetic models shows that the release rate depends strongly on the drug concentration, especially for Dmt - HBcd lyophilized inclusion complex.

Viscosity is a measurement of flowing resistance when a shear force is applied on a material [8]. Figure 4 (a) shows the variation of the shear stress *versus* the shear rate for Dmt with Bcd mixtures, and Figure 4(b) shows the same dependence, but for Dmt with HBcd mixtures. The slope of all the curves, dynamic viscosity, decreases until a minimum is reached. For this type of flow, gels seem to have a pseudoplastic or shear thinning behavior, but viscosity does not recover immediately upon the release of shear.

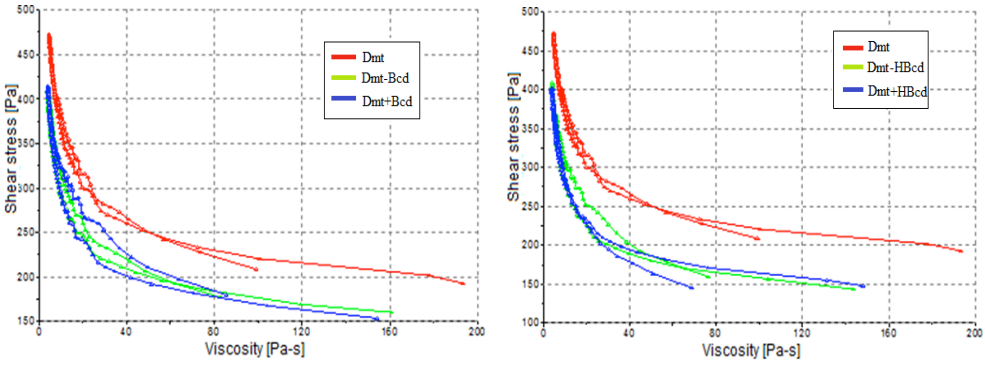


(a) (b)

Figure 4

Shear stress *versus* share rate for Dmt with Bcd mixtures (a) or Dmt with HBcd (b)

At higher shear rates the flow behaviour becomes approximately constant, reaching a limit of flow, and gel on the skin will not fall under the action of gravity. The dependence of shear stress *versus* viscosity is shown in Figure 5 (a) and (b) for all the studied semisolid dosage forms. Their behaviours seem to be viscoelastic; there are two paths for loading and unloading, signifying the energy dissipation which occurs during deformation.



(a) (b)

Figure 5

Shear stress *versus* viscosity for the inclusion complexes of Dmt with Bcd mixtures (a) or Dmt with HBcd (b)

More, once the shearing stress is stopped, the viscosity recovers over the time. There is a measurable delay in the recovery of viscosity, allowing to occur a period of leveling. Such behaviour defines the thixotropy, and can be evaluated by the hysteresis area. The thixotropy values are presented in the first column of the Table III. The lower thixotropy values are for Dmt and Dmt-HBcd gels, being about 950Pa.s. The thixotropy values of the other three gel formulations are about two times higher.

Table III

Thixotropy and Herschel–Bulkley model parameters for different Dmt gels

Gel	Thixotropy [Pa/s]	Minimum shear stress, τ_0 [Pa]	Flow consistency index, K[Pa.s^{-1}]	Flow behavior index, *n*	Correlation coefficient, R^2
Dmt	935.9	208.7	29.2471	0.4757	0.9925
Dmt- Bcd	1081	176.9	26.0684	0.4725	0.9945
Dmt-HBcd	957.8	145.2	27.0744	0.5129	0.9932
Dmt+ Bcd	1770	179.7	27.5246	0.4616	0.9915
Dmt+HBcd	1845	159.6	29.6631	0.4602	0.9912

The recorded values from the rotational rheometer RC1 were fitted by using the Herschel–Bulkley model, as follows:

$$\tau = \tau_0 + K\dot{\gamma}^n \qquad (6)$$

where τ is the share stress [Pa], τ_0 is the minimum share stress, $\dot{\gamma}$ is the share rate [s^{-1}], K is the flow consistency index [Pa.s^{-1}] and n is the flow behavior index (non-dimensional).

The parameter values for each semisolid topical dosage form are presented in the last columns of the Table III. The real and simulated ascending route dependence of shear stress *versus* shear rate for the lyophilized inclusion complexes of Dmt with Bcd or Dmt with HBcd are shown in Figure 6(a) and 6(b), respectively.

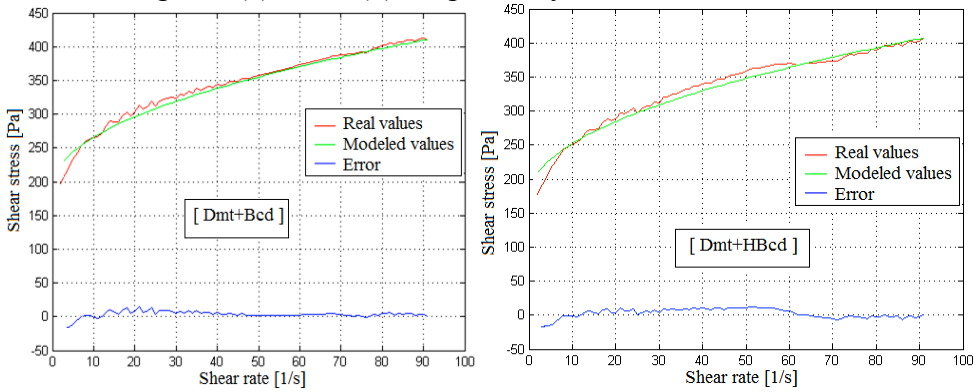


Figure 6

Real and modeled ascending route dependence of shear stress *versus* shear rate for inclusion complexes of Dmt

The interpolated data fitted accurately the measured values, the correlation coefficients between them being higher than 0.9912. The behaviour is about the same for all measured gels. At very low shear rates, the viscosity of gels is high enough, but the inclusion complexes with Cd have smaller values. The viscosities of all the gels are about the same at higher share rates. They are very small but not zero.

Conclusions

In vitro diffusion and rheological profiles were evaluated for five semisolid topical dosage forms having Dmt as active pharmaceutical ingredient, alone, in simple binary physical mixtures or in inclusion complexes with two different Cd. A significant increase of Dmt diffusivity was obtained in the case of complexation with Cd which improves skin penetration of Dmt. Also, an improvement of the release rate was noticed in the physical mixtures but not as high in the lyophilized inclusion complexes.

The rheological profiles indicated appropriate parameters of storage and use, and the thixotropy point to a little bit higher energy dissipated during deformation. The inclusion complexes of Dmt with Cd have wider prospects for topical preparations, and the influence of Bcd is the most important.

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Manuscript received: July 28th 2011