

NOVEL APPROACH FOR DETERMINATION OF BINDING CONSTANT AND THERMODYNAMIC ACTIVITY OF LIPOPHILIC DRUGS IN INCLUSION COMPLEXES

Dalia S. Shaker

Department of Pharmaceutics, Helwan University, Cairo, Egypt

في ضوء أهمية حساب كمية جزيئات الدواء التي تمر خلال الأغشية البيولوجية من خلال مركبات الدكستريانات الحلقية، تناولت الدراسة تقييم الطرق المستخدمة في حساب ثابت الارتباط معقد الدواء مع الدكسترين الحلقي وبالتالي الديناميكية الحركية للدواء. تشمل هذه الطرق تعيين معامل توزيع وانتشار الدواء بين الاوكتانول والماء وطريقة منحني الذوبان. تناولت هذه الدراسة إمكانية استخدام طريقة جديدة لحساب ثابت الارتباط والديناميكية الحركية للدواء في معقد الدواء مع الدكسترين الحلقي. تعتمد هذه الطريقة على انتشار عقار الكورتيكوستيرون كنموذج لعقار محب للدهون - في عديد جزيئات السليكون. تعتمد التجربة على حساب معاملات توزيع الدواء بين أقرص من السليكون وهي تمثل طبقة محبة للدهون ومحلول منظم الفوسفات في وجود أو غياب الدكستريانات الحلقية وأيضا في حالات التشبع وعدم التشبع باستخدام عقار الكورتيكوستيرون المشع. وقد تم تعيين ثابت الارتباط للمركب ومقارنته بالقيمة المحسوبة بطريقة الذوبان فكانت النتائج متطابقة في حين اختلفت النتائج عن تلك المحسوبة بطريقة الاوكتانول. ويعزي هذا الاختلاف إلى إمكانية تداخل جزيئات الاوكتانول الدهنية في التجويف الدهني للدكسترين الحلقي. كما تم التوصل إلى تطابق جميع قيم الديناميكية الحركية بالثلاث طرق المستخدمة وعليه أوضحت هذه الدراسات فاعلية الطريقة المستحدثة باستخدام عديد جزيئات السليكون للتعين المباشر للديناميكية الحركية لعقار الكورتيكوستيرون في المركب الإحتوائى للكورتيكوستيرون باستخدام الدكسترين الحلقي.

In light of prediction of extent of drug molecules that cross biological membranes in drug-complex system, there is a need to re-evaluate the old concept of measuring the binding constant of complexation (K_c) and consequently the drug thermodynamic

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Corresponding author E-Mail address: dalias_shaker@hotmail.com

activity (A_T) through octanol-water partition experiment or solubility technique at saturation condition. This study examined the feasibility of the silicone polymer uptake method for determination of A_T of corticosterone (CS)-as a model lipophilic drug-in hydroxypropyl beta cyclodextrin (HP β CD). Commercial medical grade silicone rubber sheets were used first in the study then replaced by prepared silicone discs to ensure the validity of CS partition data obtained in silicone/phosphate buffered saline (PBS) system in absence and presence of HP β CD. In cases of saturation and unsaturation with trace level radioactive corticosterone ($^3\text{H-CS}$), the K_c was measured. It was the same at saturation as well as lower drug concentration, and in correlation with that measured by solubility technique at saturation. However, the K_c measured by silicone method deviated from that obtained from octanol/ phosphate buffered saline partition experiment due to possible interaction between HP β CD as a complexing agent with lipophilic cavity and the organic phase (octanol). The measured A_T values were in agreement with those determined by octanol and solubility methods. This study has clearly shown the validity of the silicone polymer uptake method for the direct determination of CS thermodynamic activity in CS/ HP β CD inclusion complex.

INTRODUCTION

Previous studies^{1&2} on transdermal permeation demonstrated that the stratum corneum (SC) lipid lamellae are well represented by octanol/water system. In these studies the correlation between steroids partitioning into lipid bilayer of Hairless Mouse Skin SC and their octanol/water partition coefficients was investigated in presence of chemical permeation enhancers. The data were consistent with the view that the enhancer polar head groups locate in the polar regions of SC lipid lamellae that are represented by water saturated octanol system. In their saturated systems the drug

thermodynamic activity was unity i.e., all permeant molecules in the aqueous enhancer solutions are free as there was no kind of interaction between permeant and enhancer molecules. If a complexing agent was introduced in the system it appears to lower the thermodynamic activity of the drug.³

In order to develop drug delivery systems providing variable drug delivery rates for steroids, complexing agents such as cyclodextrins can be used. Cyclodextrins are capable of forming inclusion complexes⁴ with drugs. These non-covalent inclusion complexes can have physical, chemical and biological properties that are different from those of either

the parent drug or cyclodextrin. These complexes can improve the chemical stability and/or the aqueous solubility of drugs⁵⁻⁷.

It is important to realize that the kinetics of inclusion complex formation and dissociation between a cyclodextrin and a drug molecule is fast. The half-lives for complex formation, dissociation are much less than one second. That is, inclusion complexation is dynamic process.⁸

The thermodynamic activity or chemical potential of drugs in inclusion complexes is viewed as the principle parameter in drug permeation through biological membranes. In this study, the feasibility was examined of a novel silicone polymer-uptake method for the direct determination of corticosterone (as a model lipophilic drug) thermodynamic activity in 2-hydroxy propyl- β -cyclodextrin. This silicone polymer was used as a reference phase for calculating the complexation-binding constant and consequently quantifying the free corticosterone concentration available to cross biological membranes with different complexing agent concentrations

EXPERIMENTAL

Materials

SILASTIC®, medical grade silicone rubber sheets, 0.4 cm thickness, Dow Corning Corporation, Medical Products, Midland, Michigan, USA. Dimethylsiloxane MED-6033, two-component silicone

elastomers that blend at 1:1 ratio by weight (Nusil, Silicone Technology, Carpinterie, CA). [³H] Corticosterone (³H-CS) at >95% purity was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO) and tested for purity by methods suggested by the supplier. Phosphate buffered saline (PBS) tablets, pH 7.4, were obtained from Sigma Chemical Company (St. Louis, MO) and used as received. Nonradiolabeled corticosterone (CS) (Sigma Chemical Company) was recrystallized in absolute ethanol. Hydroxypropyl- β -cyclodextrin (HP β CD; MW~1380, MS = 0.6) was purchased from Sigma. Phenolphthalein standard solution 1 mg/ml in ethanol (Sigma Diagnostics, St. Louis, MO).

Equipment

Liquid scintillation counter, Model 1900 TR (TRI-CARB™ liquid scintillation analyzers) Packard Instrument Company, USA. HPLC system consisted of a Beckman pump, a HP 1050 series auto-sampler HPLC, with a variable wave length UV absorbance detector and a 15 cm x 4.6 mm Discovery ® C 18 column, packing particle size 4.0-4.3 mm (Supelco, Bellefonte, PA). Gravity oven, STABIL-THERM®, Model OV-12A, Illinois, USA. Autoclave, HIRAYAMA, Amerex Instruments Incorporation, Lafayette, California, USA. Mini hot plate, Model 210, VWR Scientific, Willard, Ohio, USA. Thermostatted water bath model YB-521, American Scientific Products,

McGaw Park, IL, USA. Liquid scintillation cocktail, Ultima Gold, high flash-point universal LSC-cocktail, Packard, Meriden, CT., USA. Scintillation Vials, Kimble Glass Inc., Vineland, New Jersey, USA. A series of micro-pipettes, Eppendorf 2000, variable volume, Brinkmann, VWR Scientific Products, Willard, Ohio, USA.

METHODOLOGY

Determination of corticosterone partition coefficient by Silastic® rubber sheets partitioning experiments

Prior to experiment, the SILASTIC® rubber sheets were rinsed with PBS. Exactly one gm of medical grade silicone polymer (cut into pieces of dimension < 2 mm) was added to each vial containing 1 ml of various CS concentration with or without ³H-CS added in PBS from trace levels to CS solubility (0.23 mg/ml). The vials were left for 1-6 days to equilibrate in a shaking water bath at 37°. There was no significant difference between the partitioning data determined in the experiments with 2 to 6 days. Therefore, all experiments were carried out by leaving the vials for 4 days at 37°. After equilibrium was attained, aliquots from the aqueous phase were withdrawn and analyzed for ³H-CS content by scintillation counter after dilution with 10 ml scintillation cocktail and for nonradiolabeled CS content by HPLC. Methanol (HPLC grade) / Water ratio of 66:34 was

used as the mobile phase at a flow rate of 1.0 ml/min. The ³H-CS or CS content of the silicone pieces was also determined after double extraction with absolute ethanol. The distribution of ³H-CS as well as CS molecules between silicone and aqueous solution, described by the partition coefficient was determined.

Validation of partitioning experiments after ethanol pretreatment of silastic sheets

In order to validate the partition coefficient data obtained in the previous experiment, the silicone sheets were pretreated with different procedures regarding rinsing or boiling in ethanol. Then pretreated sheets were dried and cut into pieces for partition experiments.

Pretreatment of Silastic sheets

Rinsing procedure

About 5 gm silicone rubber (cut into pieces of dimensions less than 2 mm) was rinsed with 15 ml boiling 95% ethanol for 10 sec. Almost all ethanol is rejected using disposable glass pipette and replaced with another 15 ml boiling alcohol for second rinse of about 10 sec. A third rinse with 20 ml boiling ethanol for 10 sec took place followed by three times rinse with boiling deionized water (about 15 ml for 10 sec each rinse) to remove any residual alcohol.

Boiling procedure

About 15 ml boiling 95% ethanol was added to 5 gm silicone rubber. The polymer was allowed to boil on hot plate for about 20 sec, then

removed from heater and all ethanol was removed and replaced by 15 ml boiling alcohol and again the polymer was allowed to boil for another 20 sec. This procedure was repeated for the third time with 20 ml boiling ethanol for 20 sec. After removal of all ethanol, the polymer was rinsed three times with boiling deionized water (about 15 ml for 20 sec each rinse).

Boiling procedure under drastic conditions

Five grams polymer was allowed to boil in 95% ethanol on hot plate for about 15 min (ethanol was removed and replaced by 15 ml boiling alcohol about 5 to 6 times till 15 min boiling time achieved), then all ethanol removed and the polymer was rinsed five times with boiling deionized water (about 15 ml for 60 sec each rinse).

Drying of the silicone rubber

On aluminium foil, the polymer was left to dry for 2 hr in oven at 75°, then left on desk top at room temperature for 24-36 hr.

Partition experiments were carried out with the three differently pretreated polymer samples and CS partition coefficient was calculated in each case and compared to that obtained with non pretreated silastic sheets.

Preparation of silicone polymer

Sheets of silicone were prepared by mixing equal amounts of dimethylsiloxane elastomers under vacuum. The mixture was spread onto

glass dishes and allowed to cure at 150° for 10 min in autoclave then left at room temperature for 24 hr prior to use. Discs of 7 mm diameter and 1 mm thickness were cut and weighed.

Detection of hydroxypropyl- β -cyclodextrin diffusivity in prepared silicone polymer

A colorimetric technique was used to detect the diffusivity of HP- β -CD in silicone discs. Vials containing 2 discs each in equilibrium with 2 ml 5% HP- β -CD solution in PBS, were left for 4 days. After that, the HP- β -CD content in aqueous phase was determined. The 2 ml solution in each vial was prepared for analysis by adding 500 μ L of 1.0 M Na₂CO₃ solution, followed by 200 μ L of phenolphthalein standard solution. The volume was completed to 5 ml with PBS. In the control assays the sample was replaced by an equal volume of PBS. The color intensity was immediately measured at 550 nm against the blank at 25°. The HP- β -CD concentration was calculated from a calibration curve made by using 1-2000 μ L of the HP- β -CD standard.

The prepared silicone polymer partitioning experiments with different cyclodextrin concentrations

The prepared silicone discs were placed in 2ml vials (2 discs per vial) and equilibrated with 1 ml solutions of various CS concentrations with or without ³H-CS added in PBS from trace levels to CS solubility and with

different concentrations of HP β CD present. The vials were left for 1-8 days to equilibrate in a shaker water bath at 37°. There was no significant difference between the partitioning data determined in the experiments with 2 to 8 days (data not shown). Therefore, all experiments were carried out by leaving the vials for 4 days at 37°. After equilibrium was attained, aliquots from the aqueous phase were withdrawn and analyzed for ³H-CS content by scintillation counter and for nonradiolabeled CS content by HPLC. The ³H-CS or CS content of the silicone discs was also determined after double extraction with absolute ethanol. The drug-silicone/ HP β CD partition coefficient was determined and used to calculate the complexation binding constant. The stability constant of complexation was calculated for both CS and ³H-CS separately then compared with that obtained by ordinary solubility method.

Determination of CS/cyclodextrin binding constant by solubility method

Exactly 70 mg of CS previously recrystallized using ethanol was added to 8 ml of solutions of different concentrations of HP β CD in PBS, in screw capped Pyrex culture tubes. The tubes were shaken for 96 hr at 37° in a thermostatted water bath. The tubes were then centrifuged for 15 min at 3500 rpm, 1318 g. The clear supernatant solution was filtered through Millipore® filter previously saturated with the CS solution, and

analyzed for CS concentration by HPLC. A phase-solubility diagram is constructed by plotting the total molar concentration of CS on the y-axis and the total molar concentration of cyclodextrin added on the x-axis. The stability constant (K_c) of CS/ HP β CD complexes was determined from the slope of phase-solubility diagram and the solubility of CS in PBS. The K_c value obtained by this method was compared to that obtained by the silicone uptake method in order to validate this new approach for determination of drugs thermodynamic activities in complexation.

Determination of ³H-CS/cyclodextrin binding constant by octanol/PBS partitioning method

In 13 ml screw capped vials, exactly 2 ml of octanol was added to 2 ml ³H-CS solution in PBS with and without 1 and 5% HP β CD. The experiments were carried out by leaving the vials for 4 days at 37°. After equilibrium was attained, 10 μ l aliquots from the aqueous phase as well as octanol phase were withdrawn and analyzed for ³H-CS content by scintillation counter. The partition coefficient of the radiolabeled drug between octanol and PBS and between octanol and HP β CD were determined and used for calculation of stability constant of the complexation (K_c). The value of K_c obtained by octanol/PBS partition method was compared to those obtained by silicone uptake and solubility methods.

Calculation of CS thermo-dynamic activity in HPβCD inclusion complex

The thermodynamic activity of CS in the HPβCD inclusion complex at equilibrium was calculated from data obtained by silicone uptake method, solubility technique and octanol/PBS partition.

RESULTS AND DISCUSSION

Determination of corticosterone partition coefficient in Silastic® rubber sheets /PBS system

The partition coefficient (K) of non-radiolabelled corticosterone (CS) as well as radiolabelled drug (³H-CS) was determined using organic/ aqueous system. The organic phase was represented by pieces of Silastic® rubber sheets rinsed with PBS prior to use. Silicone polymer was selected as an inert polymer for its great diffusivity for organic molecules.

In literature, thin 0.006-inch thickness polyethylene discs, named Sallee's discs, were used to measure monomer activities of fatty acids in micellar solutions.⁹ There are other reports on measuring oleic acid and cholesterol uptake using Sallee's discs.¹⁰ Details of equilibrium times and whether oleic acid and cholesterol interact in the polyethylene discs were not discussed by these investigators. However, the diffusivities for organic molecules in silicone polymer are two orders of magnitude greater than in polyethylene.¹¹ Therefore, silicone

polymer was used in this study as a suitable organic phase for corticosterone partition coefficient determinations. The distribution coefficient K of CS molecules between silicone and aqueous solution was determined according to eq. (1).

$$K = \frac{A_{SP,Eq} / Wt_{SP}}{A_{Aq,Eq} / Wt_{Aq}} \quad (1)$$

Where $A_{SP,Eq}$ and $A_{Aq,Eq}$ are the molar amounts of CS in silicone and aqueous mediums at equilibrium respectively measured by HPLC. Wt_{SP} and Wt_{Aq} are the weight in grams of silicone pieces and aqueous solution. For ³H-CS, the K can be calculated as follow:

$$K = \frac{dpm_{SP} / Wt_{SP}}{dpm_{Aq} / Wt_{Aq}} \quad (2)$$

Where dpm_{SP} and dpm_{Aq} are the total radioactivity count expressed by dpm (disintegration per minute) units in silicone and aqueous phases respectively.

The results in Table (1) show that the k value of CS in silicone/PBS system was 0.46 ± 0.022 while that of ³H-CS in the same system was around unity. This discrepancy in results was not expected, as trace level radioactive species represents the non radiolabelled drugs-behavior.¹²⁻¹⁵ Therefore, the partition experiments

were repeated after ethanol pretreatment of silastic sheets. The results in Table (2) demonstrate that for CS partition in silicone, the K value was changing with polymer pretreatment. It was 0.513 ± 0.032 after rinsing the polymer with boiled ethanol for 10 sec compared to 0.46 ± 0.022 after rinsing with PBS (Table 1). Also, the data in Table (2) show that the K value increased to 0.709 ± 0.019 and 0.865 ± 0.003 with boiling the polymer in ethanol for 20 sec and 15 min, respectively. These results show the silicone pretreatment-dependent changes in the uptake of CS by the silicone polymer. However, the ^3H -CS uptake by silicone polymer was not affected by silicone pretreatment. The K value of ^3H -CS was constant and remained close to unity in all experimental conditions (data not shown).

The reason of discrepancy with commercial silicone sheet (Silastic®) may be due to presence of silica filler, and this filler was partially or completely removed with pretreatment of the polymer with ethanol. Removing this kind of impurity increases CS diffusivity in the polymer and consequently affects the quality of data obtained in CS partitioning experiments. However, this filler has negligible effect on diffusivity of ^3H -CS in polymer due to the very small amounts of radioactive drug in the experiment and accordingly the small amounts

diffused in silicone. Therefore it was important to find a method that measure the diffusivity of drugs from trace level to saturation i.e., independent on drug concentration. Silicone discs were prepared in this study and were used to determine the binding constant of complexation between CS and HP CD. The data in Table (3) showed the constant K obtained with different pretreatment conditions of prepared silicone discs. Also the K value of CS was the same as that of trace level. The results show the validity of prepared silicone discs to determine the partition coefficient of CS at saturation as well as unsaturation.

The prepared silicone polymer partitioning experiments with different cyclodextrin concentrations

The cyclodextrin diffusivity in the prepared silicone discs was measured and there was no measurable depletion of HP CD in the aqueous layer due to the uptake of HP CD into silicone within the sensitivity limit of the colorimetric assay. This result indicates that there is no interaction between the prepared silicone polymer and the cyclodextrin used in this study. The CS silicone/HP β CD partition coefficient (K_x) in X cyclodextrin concentration was determined and used to calculate the complexation binding constant.

Table 1: Partition coefficient values of corticosterone in silicone/PBS system after rinsing SILASTIC® sheets with PBS.

Non radiolabelled corticosterone (CS)				
Concentration of CS in 1 ml aqueous phase ($C_{aq, Eq}$) ^a in mg/ml	Amount of CS in silicone polymer ($A_{sp, Eq}$)	Weight of silicone in gms (Wt_{sp})	Concentration of CS in 1 gm silicone ($C_{sp, Eq}$) ^b in mg/gm	Partition coefficient (K)*
0.201 ± 0.001	0.01 ± 0.002	0.11 ± 0.01	0.092 ± 0.001	0.46 ± 0.022
Radiolabelled corticosterone (³ H-CS)				
Dpm count in 1 ml aqueous phase calculated from 10 µl sample ($C_{aq, Eq}$) ^a in dpm/ml	Total dpm count in silicone polymer ($A_{sp, Eq}$) in dpm	Weight of silicone in gms (Wt_{sp})	Dpm count in 1 gm silicone ($C_{sp, Eq}$) ^b in dpm/gm	Partition coefficient (K)*
245061**	38116.9	0.154	247512.33	1.01
291137**	47979.39	0.16	299871.2	1.03
223444**	221165.5	0.98	225679.1	1.01

*K value was calculated by dividing b/a.

**Average dpm count of four samples, 10 µl each, to reduce pipetting errors.

Table 2: Determination of partition coefficient of corticosterone (CS) in silicone/PBS system after ethanol treatment of SILASTIC® sheets.

Rinsing SILASTIC® sheets with boiled ethanol for 10 seconds				
Concentration of CS in 1 ml aqueous phase ($C_{aq, Eq}$) ^a in mg/ml	Amount of CS in silicone polymer ($A_{sp, Eq}$)	Weight of silicone in gms (Wt_{sp})	Concentration of CS in 1 gm silicone ($C_{sp, Eq}$) ^b in mg/gm	Partition coefficient (K)*
0.2176 ± 0.0024	0.01233 ± 0.0023	0.109 ± 0.018	0.1116 ± 0.0059	0.513 ± 0.032
Boiling of SILASTIC® sheets with ethanol for 20 seconds				
Concentration of CS in 1 ml aqueous phase ($C_{aq, Eq}$) ^a in mg/ml	Amount of CS in silicone polymer ($A_{sp, Eq}$)	Weight of silicone in gms (Wt_{sp})	Concentration of CS in 1 gm silicone ($C_{sp, Eq}$) ^b in mg/gm	Partition coefficient (K)*
0.2107 ± 0.0005	0.02 ± 0.0008	0.134 ± 0.004	0.149 ± 0.039	0.709 ± 0.019
Boiling of SILASTIC® sheets with ethanol for 15 minutes				
Concentration of CS in 1 ml aqueous phase ($C_{aq, Eq}$) ^a in mg/ml	Amount of CS in silicone polymer ($A_{sp, Eq}$)	Weight of silicone in gms (Wt_{sp})	Concentration of CS in 1 gm silicone ($C_{sp, Eq}$) ^b in mg/gm	Partition coefficient (K)*
0.2086 ± 0.0009	0.0213 ± 0.0023	0.117 ± 0.005	0.181 ± 0.0007	0.868 ± 0.003

*K value was calculated by dividing b/a.

Table 3: Determination of partition coefficient of corticosterone (CS) in silicone/PBS system after ethanol treatment of prepared silicone elastomer.

Rinsing the prepared silicone polymer with boiled ethanol for 10 seconds				
Concentration of CS in 1 ml aqueous phase ($C_{aq, Eq}$) ^a in mg/ml	Amount of CS in silicone polymer ($A_{sp, Eq}$)	Weight of silicone in gms (Wt_{sp})	Concentration of CS in 1 gm silicone ($C_{sp, Eq}$) ^b in mg/gm	Partition coefficient (K) [*]
0.206 ± 0.005	0.0237 ± 0.0051	0.109 ± 0.016	0.216 ± 0.013	1.05 ± 0.09
Boiling of the prepared silicone polymer with ethanol for 20 seconds				
Concentration of CS in 1 ml aqueous phase ($C_{aq, Eq}$) ^a in mg/ml	Amount of CS in silicone polymer ($A_{sp, Eq}$)	Weight of silicone in gms (Wt_{sp})	Concentration of CS in 1 gm silicone ($C_{sp, Eq}$) ^b in mg/gm	Partition coefficient (K) [*]
0.210 ± 0.0005	0.0197 ± 0.0005	0.097 ± 0.0004	0.202 ± 0.004	0.96 ± 0.025
Boiling of the prepared silicone polymer with ethanol for 15 minutes				
Concentration of CS in 1 ml aqueous phase ($C_{aq, Eq}$) ^a in mg/ml	Amount of CS in silicone polymer ($A_{sp, Eq}$)	Weight of silicone in gms (Wt_{sp})	Concentration of CS in 1 gm silicone ($C_{sp, Eq}$) ^b in mg/gm	Partition coefficient (K) [*]
0.210 ± 0.0002	0.02 ± 0.0003	0.094 ± 0.004	0.213 ± 0.01	1.01 ± 0.05

* K value was calculated by dividing b/a.

$$K_x = \frac{A_{sp} / wt_{sp}}{A_{aq, total} / wt_{aq}} \quad (3)$$

Where K_x is CS partition coefficient in Silicone/ HP β CD system, A_{sp} is the amount in moles of CS in the silicone elastomer. $A_{aq, total}$ represents the total amount (in moles) of free CS plus solubilized or complexed CS in aqueous phase, Wt_{sp} and Wt_{aq} are the weight in grams of silicone pieces and aqueous cyclodextrin solution. The K_x value for radiolabelled drug obtained by measuring the radioactive count in both silicone and aqueous phases. After obtaining K and K_x values, the stability constant of complexation was calculated for both CS and ³H-CS separately then

compared with that obtained by ordinary solubility method.

Determination of CS/cyclo-dextrin stability constant of complexation by silicone uptake method

The CS/cyclodextrin stability constant of complexation K_c can be calculated from the measured concentration of total CS in cyclodextrin solution as well as the amount of CS in silicone polymer, using the equation:⁸

$$K_c = \frac{S_t - S_0}{S_0(L_t - (S_t - S_0))} \quad (4)$$

Where S_0 is the molar intrinsic CS solubility, S_t is the total molar CS concentration in presence of total molar HP β CD concentration (L_t).

This K_c value was later used for calculating the free CS concentration.

Determination of $^3\text{H-CS/cyclodextrin}$ stability constant of complexation by silicone uptake method

The $^3\text{H-CS/cyclodextrin}$ stability constant of complexation K_c can be calculated from the equation:

$$K_c = \frac{\left[\frac{K}{K_x} - 1 \right]}{L_f} \quad (5)$$

Where, K is $^3\text{H-CS}$ partition coefficient in silicone/PBS system, K_x is the $^3\text{H-CS}$ partition coefficient in silicone/aqueous $\text{HP}\beta\text{CD}$ system, K_c (as in eq. 4) is the stability constant of the complex and L_f is the free molar $\text{HP}\beta\text{CD}$ concentration in aqueous phase. For partitioning experiments in which only $^3\text{H-CS}$ was used $L_f = L_t$.

Calculation of the concentration of free CS in cyclodextrin

The concentration of free CS in the cyclodextrin solution is determined from the following equation:

$$C_{\text{aq,free}} = \frac{K_x}{K} C_{\text{aq,total}} \quad (6)$$

Where $C_{\text{aq,total}}$ was measured by HPLC during determination of K_x (eq. 3), also the K and K_x values were previously determined.

Calculation of the concentration of free $^3\text{H-CS}$ in cyclodextrin

The K_c value obtained from eq. (5) was used to calculate the free $^3\text{H-CS}$ in cyclodextrin solution by the following equation:

$$\text{Free}[^3\text{H-CS}], \text{aq} = \frac{\text{Total}[^3\text{H-CS}], \text{aq}}{[1 + K_c(L_t)]} \quad (7)$$

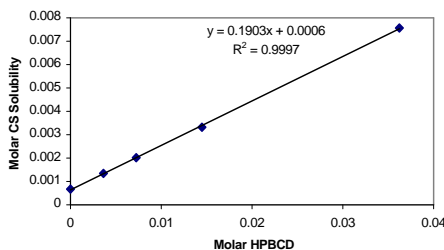


Fig. 1: Phase solubility diagram of corticosterone in $\text{HP}\beta\text{CD}$ solution.

Determination of CS/cyclo-dextrin binding constant by solubility method

A phase-solubility diagram (Fig. 1) is constructed by plotting the total molar concentration of CS on the y-axis and the total molar concentration of cyclodextrin added on the x-axis. The stability constant (K_c) of CS-cyclodextrin complexes was estimated from the slope of phase-solubility diagram and the solubility of CS in PBS (S_0). The stability constant was calculated according to equation (8):¹⁶

$$K_c = \frac{\text{Slope}}{S_0(1 - \text{Slope})} \quad (8)$$

Where S_0 is the molar intrinsic drug solubility. In case of A_L -type diagram (linear diagram), the K_c was determined from the solubility data and used for calculating the free corticosterone concentration.

Table (4) shows the values of the complexation binding constant (K_c) obtained at saturation and at trace levels in 1 and 5% HP CD with the silicone polymer partitioning method. The results show that all the data are consistent with a one-to-one complex governing the equilibrium behavior from trace levels to saturated solutions. K_c values calculated from the silicone partitioning method (357 to 369 $(M)^{-1}$) are also consistent with the value obtained from the solubility data ($\sim 350 (M)^{-1}$). This shows the appropriateness of using a single binding constant ($K_c = 360 (M)^{-1}$) for the inclusion complex from trace levels of CS to saturation in calculating the free CS concentration.

Determination of 3H -CS/ cyclodextrin binding constant by octanol/PBS partitioning method

The values of K_c obtained at trace levels in 1 and 5% HP CD with the octanol/PBS partitioning method represented in Table (5). The results show that the K_c values calculated from this method (247 to 274) deviate from those obtained from both silicone uptake and solubility techniques. This result revealed that

the old concept of organic/aqueous liquid phases i.e., octanol/water, partitioning becomes inadequate in describing drugs thermodynamic activity in complexes. In such partitioning experiments any complexing agent with a lipophilic cavity such as cyclodextrins, may interact with the liquid organic phase affecting the quality of partition data obtained.

Calculation of CS thermodynamic activity in HP β CD inclusion complex

The thermodynamic activity of CS, A_T , in the HP β CD inclusion complex at equilibrium was calculated from data obtained by silicone uptake method (data shown in Table 6), based on eq. (9):¹⁷

$$A_T = C_{sp,Eq} / C_{sp,Eq}^0 \quad (9)$$

Where C^0 is the $C_{sp,Eq}$ value at corticosterone saturation. Equation (9) follows from the convention that a solution in equilibrium with corticosterone is at unit activity. The results revealed that CS thermodynamic activity in complex measured with silicone uptake method was 0.25 and 0.9 in 1 and 5% HP β CD solutions, respectively. These values were constant at saturation (Column 3 of Table 6) as well as at trace level of radiolabeled drug (Column 5 of Table 6).

In solubility experiments the A_T was calculated from eq. (10) and demonstrated in Table (7).

$$A_T = \frac{1}{F} = \frac{1}{1 + \frac{S_x}{S_o}} \quad (10)$$

Where, F is a correction factor, S_x and S_o are the solubilities of non-radioactive CS in cyclodextrin and PBS, respectively. While A_T was calculated from Octanol/ PBS partition coefficient by eq. (11) and the values have shown in Table (8).

$$A_T = \frac{1}{F} = \frac{1}{1 + \frac{K_x}{K_o}} \quad (11)$$

Where K_o is the Octanol/ PBS partition coefficient of CS in absence of cyclodextrin while K_x is the Octanol/ PBS partition coefficient of

CS at x cyclodextrin concentration. The results shows that the drug thermodynamic activity was constant for each cyclodextrin concentration, in all measuring methods revealing the validity of silicone polymer to measure lipophilic drugs thermodynamic activity. The discrepancy obtained in binding constant value measured by octanol/PBS partition method (Table 5) was due to interaction between cyclodextrin and octanol. However, this deviation in Kc was so small that it has no effect on CS- A_T calculation (Table 8). May be this will not be the case with highly lipophilic drugs that have higher Kc values in cyclodextrins ($Kc \geq 1000$).

Table 4: Binding constant values determined from silicon uptake method that used to calculate the free corticosterone concentration in different cyclodextrin solutions.

Corticosterone Concentration	Cyclodextrin Concentration % w/v	Binding Constant Kc Calculated from Silicone/ PBS Partition Coefficient (M) ⁻¹
Corticosterone saturated solution: 0.23 mg/ml	1	373.4* ± 18 (n = 9)
	5	367.8* ± 15 (n = 8)
Trace level corticosterone ³ H-CS	1	357.1** ± 12 (n > 6)
	5	369.1** ± 20 (n > 6)

*Kc values calculated by Eq. (4)

** Kc values calculated by Eq. (5)

^a Kc determined from the solubility data and calculated by eq. (8) was 350 (M)⁻¹.

Table 5: Octanol/ PBS partition coefficient and binding constants used to calculate the free ^3H -CS concentration in different cyclodextrin solutions.

Cyclodextrin Concentration (% w/v)	^3H -CS Octanol/ PBS Partition Coefficient (K_x)	Free Molar Cyclodextrin Conc. L_f^*	Binding Constant Kc Calculated from Octanol/ PBS Partition Coefficient $^{**}(\text{M})^{-1}$
0	33.28 ± 0.02 (K)	-----	-----
1	11.19 ± 0.01	0.0072	274.17 (n = 5)
5	3.36 ± 0.01	0.036	247.35 (n = 6)

* For ^3H -CS partition experiments $L_f \cong$ the total molar cyclodextrin concentration.

** Determined by Eq. (5) and used to calculate the free radioactive CS in presence of cyclodextrin.

Table 6: Corticosterone (CS and ^3H -CS) thermodynamic activity values in HP β CD inclusion complex determined from silicon uptake method.

Cy-Conc (% w/v)	Concentration of CS in 1 gm silicone ($C_{\text{sp,Eq}}$) in mg/gm	CS thermodynamic activity* (A_T)	Dpm count in 1 gm silicone ($C_{\text{sp,Eq}}$) in dpm/gm	^3H -CS thermodynamic activity* (A_T)
0	0.213 ± 0.01 ($C_{\text{sp,Eq}}^0$)	1	257687.5 ± 38128 ($C_{\text{sp,Eq}}^0$)	1
1	0.053 ± 0.002	0.248	64421.8 ± 7782	0.25
5	0.019 ± 0.000	0.089	22934.2 ± 2770	0.089

* Determined by Eq. (9).

Table 7: Corticosterone (CS) thermodynamic activity values in HP β CD inclusion complex determined from solubility technique.

Cyclodextrin Concentration (% w/v)	CS solubility (mg/ml)	Correction factor F^*	CS thermodynamic activity (A_T) = $1/F$
0	0.23 ± 0.001 (S_0)	-----	1**
1	0.69 ± 0.01	4.0	0.25
5	2.30 ± 0.01	11.2	0.089

* Determined from Eq. (10)

** At saturation A_T is unity.

Table 8: Corticosterone (CS) thermodynamic activity values in HP β CD inclusion complex determined from octanol/PBS partition.

Cyclodextrin Concentration (% w/v)	³ H-CS Octanol/ PBS Partition Coefficient (K _x)	Correction factor F*	CS thermodynamic activity (A _T) = 1/F
0	33.28 ± 0.02 (K)	-----	1
1	11.19 ± 0.01	3.97	0.25
5	3.36 ± 0.01	10.9	0.091

* Determined from Eq. (11).

Conclusion

The objective of this research is to investigate a direct method for calculation of free drug that affect permeability behavior with complexation. As revealed from the present study it can be concluded that; silicone uptake method is valid to calculate the binding constant of complexation (K_c) at saturation as well as lower drug concentration represented by radiolabelled drug.

Silicone as lipophilic inert solid phase mimic the behavior represented by biological membranes or stratum corneum (SC) as the amount of drug partitioned in this polymer represent what will be actually partitioned in phospholipids bilayer of membranes and SC in presence or absence of complexing agents. In contrast to octanol/water liquid system in which a complexing agent with lipophilic cavity can interact with the organic phase (octanol) leading to result variation in partition coefficient data. In contrast to solubility technique that applied only at saturation and with A_L solubility type.

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