The study of the effects of surfactants on Ethylcellulose microspheres containing Salbutamol Sulphate

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Abstract

Microspheres containing Salbutamol sulphate was prepared by emulsion solvent evaporation technique using two types of surfactants, Tween 80 (polysorbate 80) and Span 80 (sorbitan monooleate 80). Infrared Spectroscopy, Differential Scanning Colorimetry and X-Ray Diffraction Spectroscopy studies were carried out to study whether the surfactants have any impact on the physicochemical properties of the microspheres. Scanning Electron Microscopy was done to study the surface topography of the microspheres. When Span 80 was used, the microspheres were smaller in size as compared to those obtained using Tween 80 while there was a higher release rate when Tween 80 was used.

Key Words: Tween 80, Span 80, Microspheres, Salbutamol sulphate, emulsion solvent-evaporation technique.

Introduction

Microencapsulation has been used as one of the methods to deliver a drug in a controlled fashion. It provides a means to modify and retard the drug release. Due to their small particle size, they are widely distributed throughout the gastrointestinal tract and this potentially improves drug absorption and reduces side effects related to localized buildup of irritating drugs against the gastrointestinal mucosa [1].
Several methods were developed for the preparation of microcapsules and emulsion solvent evaporation method is one of such methods and can be used to encapsulate both water soluble and water insoluble drugs. In microencapsulation by solvent evaporation method, surfactants play an important part in the final characteristics of the microcapsules. Tween 80 (polysorbate 80) and Span 80 (sorbitan monooleate) are two of the most commonly surfactants used interchangeably by different authors. The present study aims to rationalize their use by preparing Salbutamol sulphate microspheres using both types of surfactants and study their effects on different characteristics of the microspheres.

Salbutamol sulphate is a relatively selective β2 adrenoceptor agonist used as a first line drug in asthma. It is given orally or by inhalation and is readily absorbed in the gastrointestinal tract [2]. It is subjected to first-pass metabolism in the liver and possibly in the gut wall. Plasma half-life is about 4-6 hours. Therefore it is suitable for formulation in to an oral sustained release dosage form for 12-24 hours duration of action.

Materials and Methods

Salbutamol sulphate IP (Ducbill drugs, Kolkatta, batch no. 20050340), ethylcellulose (22 cps grade determined at 80:20 Toluene:Ethanol, Wilson Brothers, Mumbai), Tween 80 (Rankem, New Delhi, batch no. R242K04), Span 80 (CDH, Mumbai, batch no. 02128), were obtained and all other chemicals and reagents used were of analytical grade.

Infrared Spectroscopy

IR spectra of Salbutamol sulphate (pure drug and microspheres) were recorded using Perkin-Elmer model 883 IR-spectrophotometer between the ranges of 500 to 4000 cm^{-1}. The resultant spectra were then compared with standard reference (IP 1996) and observe for any type of deviation from the standard.

Differential Scanning Calorimeter Analysis (DSC)

DSC thermogram of the pure drugs and the microspheres were recorded with a differential scanning calorimeter (Universal V2.5H TA Instrument) from 20 to 550 ºC at a heating rate of 20 ºC/minute.

X-Ray Diffraction Spectroscopy (XRD)

X-Ray Diffraction Spectrum of the pure drug and microspheres were recorded with Phillips PW 1830 X-ray generator fixed with PW 1710 diffractometer (Phillips Industrial & Electro-acoustic Systems Division, Almelo, The Netherlands). The XRD was performed at the angle between 5-60 ° (2θ).

Preparation of microcapsules

The microspheres were prepared by emulsion solvent evaporation technique using the formulation as shown in Tables 1 and 2. In this method 900 mg of ethylcellulose was dissolved in 15 ml of acetone and a given amount of the drugs were dispersed in it to make different drug to polymer ratio of 1:1, 1:1.5, 1:2 and stirred for about 10 minutes. Then the polymer drug dispersion was poured into 50 ml of liquid paraffin (light) containing varying concentrations of dispersing agents. The whole system was then stirred for about 4 hours at 900 RPM. After stirring process is over the liquid paraffin (light) was decanted off and the microcapsules formed were collected and washed with Cyclohexane to completely remove the remaining oil and dried at 50 ºC in Vacuum drier (NSW, India) for 6 hours and collected for further studies.

Particle size determination

The particle size of the microspheres was determined by microscopic method [3]. For each batch of the microspheres, 100 particles were counted and done in triplicate.

Table 1: Formulation of Salbutamol Sulphate microspheres with Tween-80

Formulations	**A1**	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
Drug:Polymer	1:1	1:1	1:1	1:1.5	1:1.5	1:1.5	1:2	1:2	1:2
Tween 80 (%)	0.2	0.6	1.0	0.2	0.6	1.0	0.2	0.6	1.0

Table 2: Formulation of Salbutamol Sulphate microspheres with Span-80

Formulations	**D1**	**D2**	**D3**	**E1**	**E2**	**E3**	**F1**	**F2**	**F3**
Drug:Polymer	1:1	1:1	1:1	1:1.5	1:1.5	1:1.5	1:2	1:2	1:2
Span 80(%)	0.2	0.6	1.0	0.2	0.6	1.0	0.2	0.6	1.0

Drug entrapment efficiency

The amount of Salbutamol sulphate present in the microsphere was determined by extraction in distilled water [4]. 50 mg of the crushed and powdered microsphere was taken and extracted in 50 ml of distilled water and stirred for 15 minutes at 1500 RPM. The solution was filtered and after suitable dilutions the content of Salbutamol sulphate was determined spectrophotometrically at 276 nm (U-2001, Hitachi).

$$\text{Drug Entrapment Efficiency} = \frac{\text{Experimental Drug Content}}{\text{Initial Drug Content in the Formulation}} x100$$

In vitro drug release study

The in-vitro release study of the microsphere was carried out using USP rotating basket method at 50 rpm at 37 ºC. Dissolution study was performed in Phosphate buffer pH 7.4 taking 900 ml for each study. 50 mg of the microsphere was taken and samples were taken at a predetermined time intervals up to 12 hours and Salbutamol sulphate content was determined by UV spectrophotometer at 276 nm.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy was done to characterize surface topography of the microspheres. Photomicrograph of the microspheres before and after the release of drugs was taken (Hitachi S-3600N, Japan). The quality of the microspheres (with respect to

surface properties) and the nature and size of pores developed on the surface can be studied. The changes that occur during in-vitro dissolution studies may have implications to the performance of the microspheres.

Release Kinetics

Data obtained from in vitro release studies were fitted to various kinetic equations to find out the mechanism of drug release from the ethylcellulose microsphere. The kinetic models used were:

(1) $Q_t = k_o t$ (zero-order equation)

(2) $ln\ Q_t = ln\ Q_0 - k_1 . t$ (first-order equation)

(3) $Q_t = K .S. \sqrt{t} = k_H . \sqrt{t}$ (Higuchi eq^n based on Fickian diffusion)

Where, $\boldsymbol{Q}$ is the amount of drug release in time t, $\boldsymbol{Q_0}$ is the initial amount of drug in the microsphere, S is the surface area of the microcapsule and $\boldsymbol{k_o}$, $\boldsymbol{k_1}$, and $\boldsymbol{k_H}$ are rate constant of zero order, first order and Higuchi rate equations respectively. In addition to these basic release models, there are several other models as well. One of them is Peppas and Korsenmeyer equation (power law) [5,6].

$$M_t / M_\infty = k \cdot t^n$$

Where M_t is the amount of drug release at time t and M_∞ is the amount release at time t = ∞, thus M_t / M_∞ is the fraction of drug released at time t, k is the kinetic constant, and *n* is the diffusion exponent which can be used to characterize both mechanism for both solvent penetration and drug release. Determining the correlation coefficient assessed fitness of the data into various kinetic models. The rate constants, for respective models were also calculated from slope.

Results and Discussion

The physicochemical stability and compatibility studies performed through infrared spectroscopy (Figure 1), Differential Scanning Colorimetry (Figure 2) and X-Ray Diffraction spectroscopy (Figure 3) all shows that both types of surfactants do not cause any large shift or deviation in the spectra of the drugs when formulated into microspheres.

Scanning electron microscopy of drug-loaded ethylcellulose microspheres (Figure 4) shows that the microspheres posses a rough and rugged surface. The surface contains some crystals deposited in it, which probably is a drug that is required for initial burst release. The micrograph taken after 12 hours release studies also reveals porosity developed at the surface. The surface porosity is crucial for drug release in microspheres prepared with ethylcellulose. Since the polymer is not biodegradable, the release of the drugs from microspheres takes place by dissolution and diffusion through these pores. Ethylcellulose allows water to permeate through its surface without itself dissolving in it.

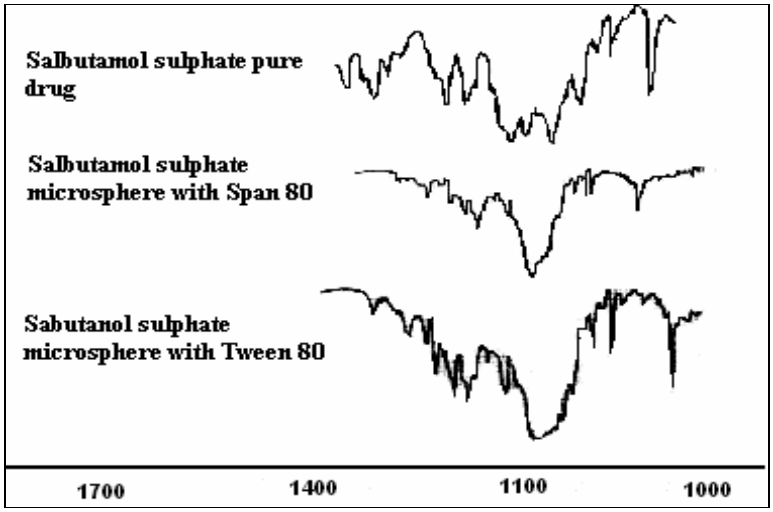

Figure 1: IR Spectra of Salbutamol sulphate drug, Salbutamol sulphate microspheres prepared using Tween 80 and Span 80

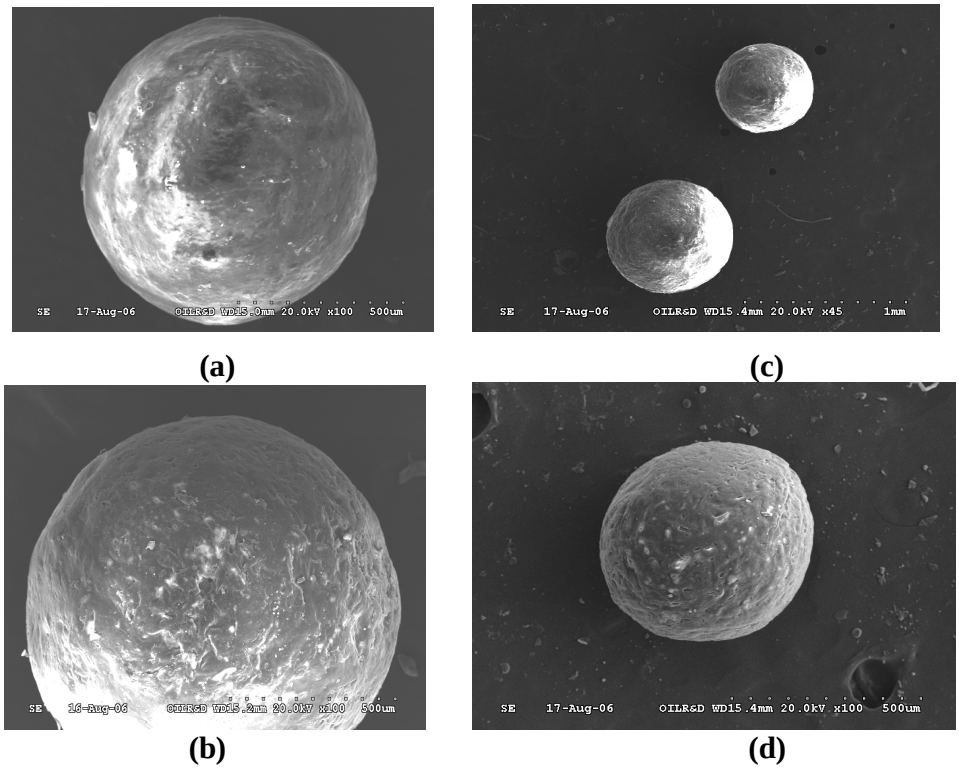

Figure 2: Scanning electron micrograph of Salbutamol sulphate microspheres prepared from Tween 80 and Span 80 (a) Tween 80 before drug release (b) Tween 80 after 12 hours drug release and its magnification (c) Span 80 before drug release (d) Span 80 after 12 hours drug release.

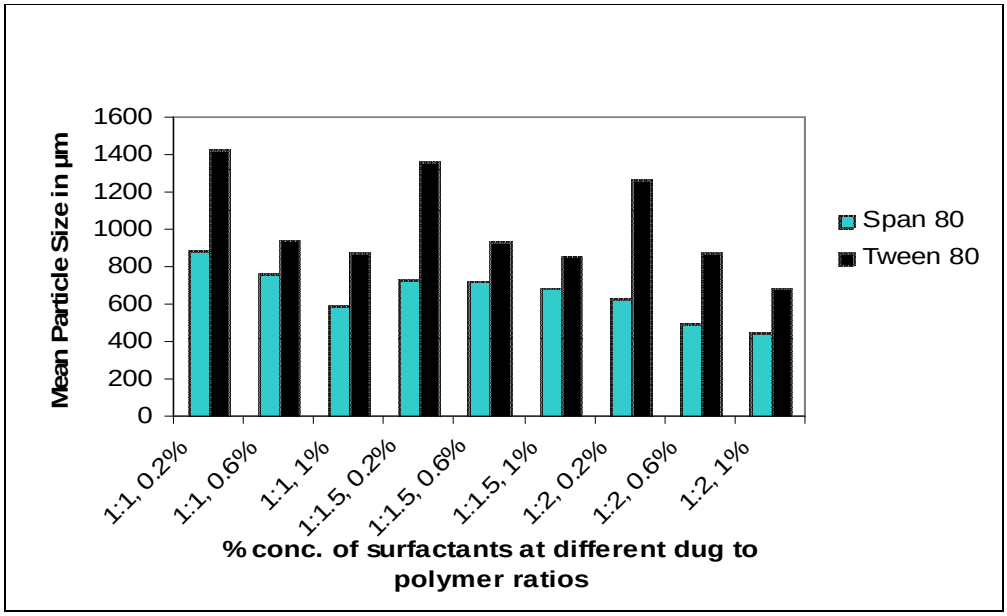

Figure 3: Comparison of mean particle size of the microspheres

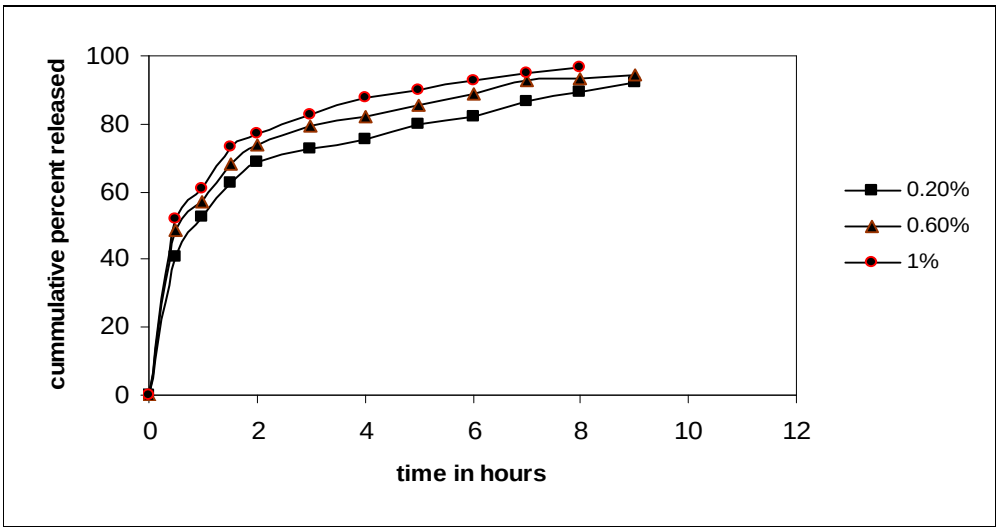

Figure 4: Effect of Tween 80 concentration on drug released (D:P =1:1)

The micrographs show that porosity developed but structure is retained as 12 hours drug release study was carried out.The mean particle size of the formulations were found to be in between 500 nm and 1400 nm as shown in Table 2, the mean particle size distribution was found to be affected by variables taken (types and concentrations of surfactants, polymer to drug ratio).

Two types of surfactants used have an influence on the particle size distribution of the microspheres (Figure 5). The hydrophobic surfactant Span 80 (Sorbitan monooleate, HLB 4.3) is found to produce smaller particle size microspheres compared to hydrophilic surfactant Tween 80 (Polyoxyethylene 20 sorbitan monooleate, HLB 14.9). Span 80 is oil soluble and produces a stable emulsion when the dispersion medium is oil. This may explain why smaller particle sizes are obtained with span 80. The concentration of surfactant/dispersing agents also affects the particle size. For both types of surfactants used, the higher concentration of surfactant resulted in production of smaller particle size. This is due to better stabilization of internal droplets with increase of surfactant concentration preventing coalescence. Also when more amount of surfactants are added, there is an accelerated dispersion of microcapsules in the microencapsulation system [7].

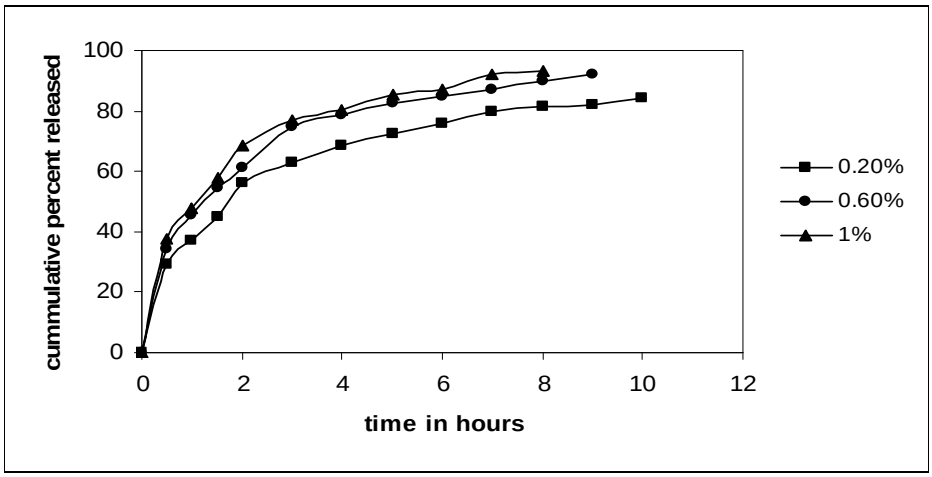

Figure 5: Effect of Span 80 concentration on drug release (D:P =1:1)

Variations in drug to Polymer ratio also affect the mean particle size distribution. Constant amount of polymer was taken and dissolved in the solvents, and the amount of drug added is varied. As the Polymer to drug ratio decrease, larger particle size is obtained. This is due to the increase in viscosity of the polymer-drug dispersion as the ratio of polymer: drug is increased to 1:1. There are also similar reports available [8].

The entrapment efficiency was determined at phosphate buffer of pH 7.4. Higher percentage entrapment was found when the percentage of surfactant was increased from 0.2 % to 1 %. This is true in both types of surfactants used. The effect of increased in polymer concentration on drug entrapment is not indicated significantly in the results.

The in-vitro release studies reveal that as the concentration of the surfactant is increased at constant polymer to drug ratio, the rate and amount of drug release is also increased. This is due to the increase in wettability and better solvent penetration as the surfactant is increased. This effect is observed in both types of surfactants taken. Increase in surfactant concentration may also let to the increase in amount of drugs deposited at the surface. The type of surfactant taken also affects the in-vitro release behavior of the microspheres (Figures 6 and 7). Two types of surfactants Tween 80 and Span 80 are taken. In vitro

release study in Phosphate buffer pH 7.4 shows that the rate of drug release was faster in case of hydrophilic surfactant Tween 80. This is due to the hydrophilic nature of the surfactant [9], also reported similar types of finding on ethylcellulose films. Microspheres prepared using Span 80 are expected to release the drugs faster than microspheres prepared using Tween 80 due to their smaller particle size. But increase in surface area available for drug release is not effective enough as compared to hydrophilic nature of the microspheres to increase its release. But within the same type of surfactant, increase in surfactant concentration led to reduced particle size, increase surface area and increase drug release.

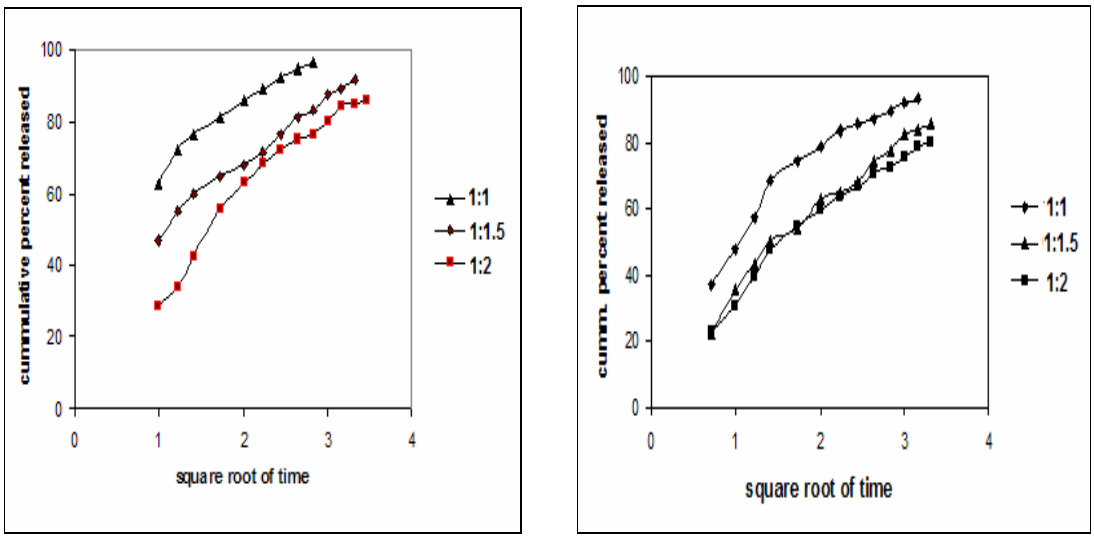

Figure 6: Higuchi plot of Salbutamol sulphate from ethylcellulose microspheres of different drug to polymer ratio. Constant amount of (a) Tween 80 (1%) and (b) Span 80 (1%)

The effect of drug to polymer concentration on in vitro release was studied. Drug to ethylcellulose ratio taken were 1:1, 1:1.5 and 1:2. The increase in polymer concentration resulted in decrease of drug release. The decrease in drug release rate with increase in polymer concentration is due the increase in wall thickness as the polymer concentration is increased which results in a longer diffusion path [8].

The in-vitro release data were fitted into various postulated kinetic models (Tables 4 and 5). The release of Salbutamol sulphate from the microspheres exhibit diffusional characteristics and closely follows Higuchi Model and also highly correlated with first-order release model.

Results of experiments showed that the amount and types of surfactants have significant effects on the performance of the microspheres when microspheres are prepared by solvent evaporation method. Span 80 was found to produces good spherical microspheres but of smaller size compared to microspheres prepared using Tween 80. Drug release was found to be slower in case of microspheres prepared with Span 80. The rate of drug release can be describe by Higuchi equation and also closely related to firs-order equation (Figures 9 and 10).

Table 4: Kinetic Table for Salbutamol Sulphate Microspheres [Span 80(1 %)]

Formulation (D:P)	Zero-Order Model		First-Order Model		Higuchi Model		Korsenmeyer-Peppas Model	
	r^2	K_o	r^2	K_1	r^2	K_h	r^2	n
1:1	0.6947	6.6512	0.9576	0.114	0.8995	26.552	0.994	0.4961
1:1.5	0.8357	6.2262	0.9745	0.0698	0.9762	24.743	0.9878	0.3172
1:2	0.8078	5.7496	0.9570	0.0589	0.9616	23.115	0.9979	0.2936

Table 5: Kinetic Table for Salbutamol Sulphate Microspheres [Tween 80(1%)]

Formulation (D:P)	Zero-Order Model		First-Order Model		Higuchi Model		Korsenmeyer-Peppas Model	
	r^2	K_o	r^2	K_1	r^2	K_h	r^2	n
1:1	0.607	8.0134	0.9491	0.1841	0.9827	26.485	0.9588	0.5622
1:1.5	0.7493	5.7121	0.9652	0.0808	0.9787	23.489	0.9827	0.5034
1:2	0.8403	5.0517	0.9705	0.068	0.9709	25.092	0.8678	0.3173

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