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The effect of different concentrations of surface active agent on the absorption of sulphamethoxazole and trimethoprim from rabbit ileum

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الخلاصة

يستخدم الترايمثيبريم (TMP) والسلفاميثوكزازول (SMX) معاً على نطاق واسع لعلاج العديد من الالتهابات. اجريت هذه الدراسة لمعرفة مدى تاثير زيادة تراكيز عوامل الشد السطحي (SAA) المستخدمة في المعلقات على امتصاص هاتين المادتين من امعاء الارنب المعزولة بدرجة حرارة 37 ْم و pH 7.4. ان عوامل الشد السطحي المستعملة كانت: صوديوم لوريل سلفات (SLS) , توين (Tween 80) , و بولي اثيلين كلايكول (PEG400 و PEG4000) بتراكيز (0.1%w/v, 1%w/v, 2% w/v, 15% w/v) لكل منهم (ملاحظة: تركيز 15 % w/v اختير لاغراض البحث وليس لاستعماله كجزء من تركيبه) . وجد ان متصاص هذين الدواءين يقل كلما ازدادت تراكيز عوامل الشد المختلفة .

ABSTRACT

A combination of sulphamethoxazol SMX and trimethoprim TMP is widly used in the treatment of various systemic infections . This investigation was carried out to see the effect of increasing concentration of surface active agents (SAA) used in suspensions on the absorption of both drugs from isolated rabbit ileum . The study was carried out at PH 7.4 and at 37°C . The SAA chosen were tween 80, sodium laurylsulphate (SLS), polyethylene glycole (PEG400), and PEG4000 at a concentrations of 0.1%w/v , 1%w/v, 2%w/v and 15% w/v (Note : 15% was chosen for the purpose of the investigation not as a concentration for formulation). It was found that the absorption of both drugs decreased as a function of increasing the concentration of the different SAA.

INTRODUCTION

The introduction of trimethoprim in combination with sulfamethoxazole constitutes an important advance in the development of clinically effective antimicrobial agent and represents the practical application of a theoretical consideration , that is if two drugs act on sequential steps in the pathway of an obligate enzymatic reaction in bacteria the result of their combination will be synergestic (1,2) .Both drugs are very slightly soluble in water and are rapidly and almost completely absorbed from the GIT and the peak concentration in circulation occurs about 1-2 hrs after an oral dose (3) . Since TMP and SMX are poorly soluble drugs in a suitable solvent, then formulation as a suspention is usually required.

An aqueous suspention is a usual dosage form for administering an insoluble or poorly soluble drug (4). Some insoluble drugs may be easily wetted and will disperse easily through out the aqueous phase with minimum agitation, other drugs are not penetrated easily by the vehicle. To make powder more penetrable by the dispersion medium, surface active agent, hydrophilic polymers, and co-solvents are employed as wetting agents (5) . Since many exciepients can alter the physical properties different dosage forms (6,7), surfactants in general cannot be assumed to be an inert excipient as they have been shown to be capable of either increasing , decreasing , or exerting no effect on the transfer of drugs across biological membranes (4) . This investigation is concerned with the effect of increasing concentrations of SAA on the absorption of TMP and SMX. Four different SAA were chosen and four concentrations were selected. The in vitro absorption study was carried out on rabbit ileum.

MATERIALS AND METHODS

Materials:

1. Spectrophotometer (piunicam).
2. Dissolution apparatus (Erweka DT6 Germany).
3. Trimethoprim powder (SDI).
4. Sulphamethoxazol powder (SDI).
5. Surfactants, tween 80, sodium laurylsulphate, polyethelene glycol 400 and 4000.
6. Asolute ethanol (BDH) Limited Pool England.
7. Phosphate buffer pH 7.4 and 7.8.
8. Rabbits.

1. Preparation of drug stock solution:
   A 0.2% stock solution of TMP was prepared by dissolving 200 mg of TMP powder in a total volume of 100 ml of [25% alcohol in buffer pH 7.8], this will give a concentration of 2 mg/ml of the drug. While a 0.25% stock solution of SMX was prepared by dissolving 250 mg of SMX powder in a total volume of 100 ml of [25% alcohol in buffer pH 7 .8] , this will give a concentration of 2.5 mg/ml of the drug .

2. Preparation of a mixture of drug with different concentration of SAA:
   For each drug , 25 ml of stock solution was mixed with 0.05 ,0.5 ,1 , and 7.5 gm of tween 80 ,SLS ,PEG 400 , PEG 4000 (1ml of tween 80 = 1.08 gm ,1ml of PEG400= 1.12 gm) then diluted to 50 ml by buffer pH 7.8 to get solutions containing 0.1 %w/v, 1 %,w/v 2 %w/v, and 15 %w/v respectively .

3. Preparation of rabbit ileum:
   After secrifying the animal, the ileum was divided into small pieces [5 cm in length] and kept in ringer solution at – 20 C until used .

4. Invitro absorption study:
   The dissolution apparatus was used, and 4 ml of the drug was injected inside the ileum and the pieces were fixed on the basket which was immersed in a vessel containing 500 ml of phosphate buffer PH 7.4. The dissolution was carried out at 37°C, stirring speed of 100 r.p.m according to USP method. Triplcate test samples of 5 ml were taken every 30 min. upto 3.5 hr and replaced by 5ml buffer immediatly. The concentration of drug was determined spectrophotometrically at λmax 275 nm for TMP, and λmax 255 nm for SMX.

5. Statistical analysis:
   1. Correlation coefficient [r] was used to test the relation between two parameters.
   2. Analysis of variance [ANOVA] was used for the comparison among multiple figures, a P value of less than 0.05 was considered to be significant.

Results and Discussion

Table 1.shows concentrations of SMX absorbed in (ug/ml) over a 3.5 hr.period, and Fig. 1-4 shows that increasing the concentration of tween 80 and PEG 4000 surfactants above the concentration of 1% cause a decrease in the amount of drug absorbed, and increasing surfactant concentration above 2% for SLS and PEG 400 (fig.2, 3) also shows a decline in the amount of drug absorbed.

Table 2.shows concentrations of TMP absorbed in (ug/ml) over a 3.5 hr. period. A decrease in the absorption above 2% concentration for surfactants tween 80, PEG 400 and PEG 4000 is shown by (fig. 5, 7, 8), while SLS in a concentration above 1% cause a decrease in absorption of the drug (fig. 6). From the above results we can see that there is an initial enhancement in the absorption at certain concentrations of surface active agents above which there is a marked decrease in the absorption. It has been shown that most surfactants interact with the absorbing membrane to bring about an enhanced permeability which facilitate the penetration of some dissolved drugs .

This effect becomes particularly apparent when the surfactant is below or just at its critical micelle concentration (CMC) in the drug solution, but it can be decreased and even reversed when CMC is suppressed, this latter effect is assumed to be due to solubilising of drug molecules in the surfactant micelle leading to unabsorbed complex, and this agreed with reported data (8, 9, 10, 11), and this may effect the bioavailability of TMP and SMX from its oral dosage form since the bioavalability of some drugs can be reduced by the presence of excipients within the dosage forms , an example of complexes that reduce drug bioavalability are those between amphetamine and sodiume carboxymethyl cellulose , and between phenobarbitone and PEG 4000 . Complexation between drug and excepients probably occurs quite often in liquid dosage forms (4). Surfactant moreover can potentially disrupt the integrity and function of a biological membrane such an effect would tend to enhance drug penetration and hence absorption across the gastrointestinal barriers. Inhibition of absorption may occur as a consequence of a drug being incorporated into surfactant micelles. If such surfactant micelle are not absorbed, which appears usually to be the cause, then solubilization of a drug may result in reduction of concentration of [free] drug in solution in the gastrointestinal fluid that is available for absorption. As a conclusion, this study showed that the absorption of poorly soluble drug sulfamethoxazol and trimethoprim can be improved by adding any of the surface active agent used in this work at a concentration below or just at their critical micelle concentration, and this may help in the formulation of a successful oral dosage form contaning combination of these two drugs, which will be effective in the treatment of many systemic infections.

Conc. of S.A.A		Time (hr)					
		0.5	1	1.5	2	2.5	3.5
0%	T. 80	0.5	1.0	1.6	2.4	3.0	4.5
	SLS						
	PEG 400						
	PEG 4000						
0.1%	T. 80	1.4	3.9	7.3	10.1	12.9	17.0
	SLS	1.7	4.0	6.6	9.2	11.6	14.5
	PEG 400	1.2	3.0	5.8	8.0	10.2	15.2
	PEG 4000	0.8	1.4	2.6	4.1	5.9	9.1
1%	T. 80	2.1	4.8	8.4	11.0	13.6	17.9
	SLS	2.9	7.2	11.8	15.9	19.5	22.5
	PEG 400	1.6	3.2	5.5	7.6	9.3	12.6
	PEG 4000	1.3	3.4	5.3	7.5	9.8	15.0
2%	T. 80	1.7	4.0	6.5	8.9	10.7	15.8
	SLS	3.1	7.2	11.5	15.2	18.0	23.0
	PEG 400	1.1	2.9	4.7	5.5	8.4	14.6
	PEG 4000	1.0	3.8	6.1	8.3	10.2	13.5
15%	T. 80	0.2	1.7	3.3	4.8	6.2	9.7
	SLS	2.7	5.2	6.1	9.4	13.8	18.8
	PEG 400	1.1	2.3	3.3	4.9	6.4	8.9
	PEG 4000	0.6	1.3	2.2	3.0	4.1	5.9

Table 1. The change in concentration absorbed of SMX - µg/ml- with time (hr) in solutions containing different SAA with different concentrations

Conc. of S.A.A		Time (hr)					
		0.5	1	1.5	2	2.5	3.5
0%	T. 80	0.0	2.2	5.4	5.9	7.0	8.5
	SLS						
	PEG 400						
	PEG 4000						
0.1%	T. 80	0.0	0.8	3.4	7.6	10.8	14.8
	SLS	0.13	1.0	2.6	3.4	4.7	7.8
	PEG 400	0.4	2.2	4.2	7.2	10.2	15.7
	PEG 4000	1.4	3.8	6.7	9.0	11.8	16.4
1%	T. 80	0.0	0.6	4.0	7.6	10.0	15.0
	SLS	0.0	0.7	3.1	5.7	7.3	11.5
	PEG 400	0.2	1.6	3.2	5.3	8.0	16.2
	PEG 4000	0.5	3.0	5.3	7.5	9.5	12.2
2%	T. 80	0.0	2.4	8.0	11.0	14.7	21.2
	SLS	0.2	0.6	1.6	2.7	3.6	7.0
	PEG 400	2.5	5.8	9.9	12.7	15.6	21.0
	PEG 4000	0.4	4.0	6.7	10.0	12.1	16.2
15%	T. 80	0.0	0.4	0.6	2.3	3.4	6.9
	SLS	2.0	3.8	1.9	2.4	3.5	7.2
	PEG 400	0.4	2.2	3.5	5.3	6.8	9.7
	PEG 4000	0.0	0.0	0.0	0.3	1.0	3.5

Table 2. The change in concentration absorbed of TMP - µg/ml - with time (hr) in solutions containing different SAA with different concentrations.

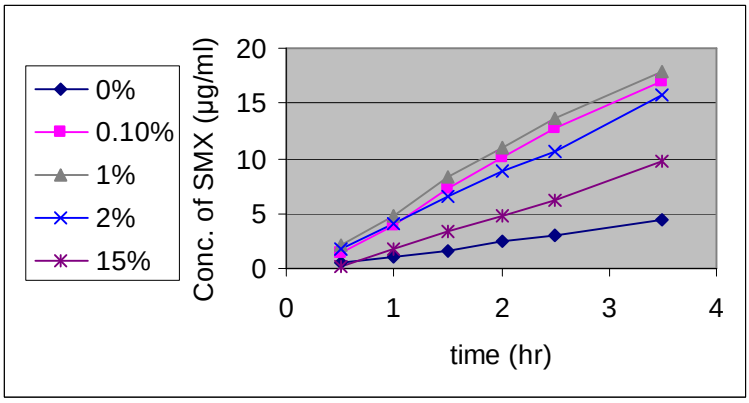

FIGURE 1. Effect of different concentrations of Tween 80 on the absorption of SMX from rabbit ileum.

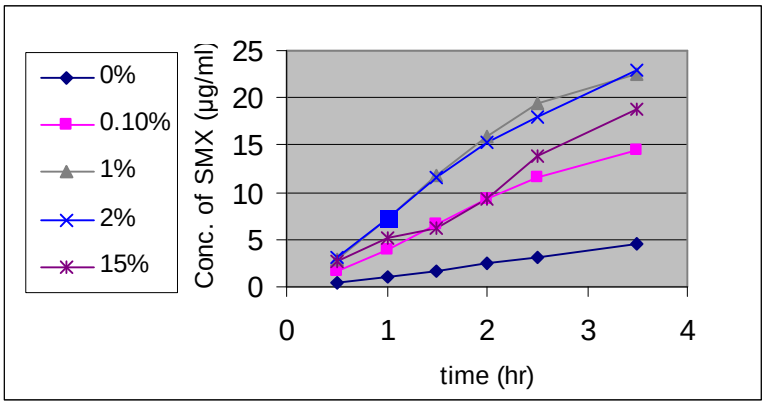

FIGURE 2. EFFECT OF DIFFERENT CONCENTRATIONS OF SLS ON THE ABSORPTION OF SMX FROM RABBIT ILEUM

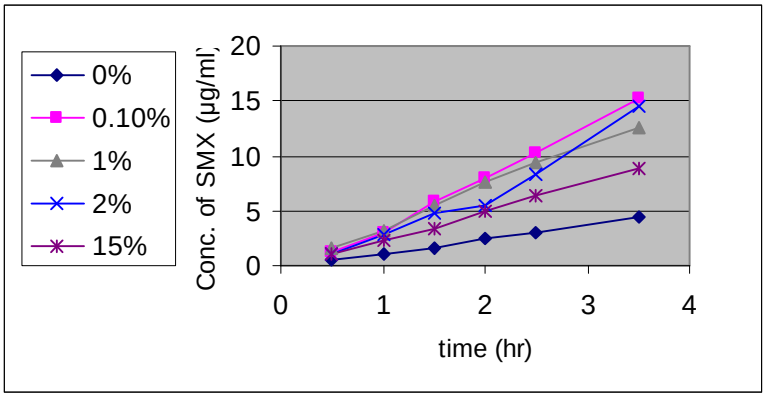

FIGURE 3. EFFECT OF DIFFERENT CONCENTRATIONS OF PEG 400 ON THE ABSORPTION OF SMX FROM RABBIT ILEUM

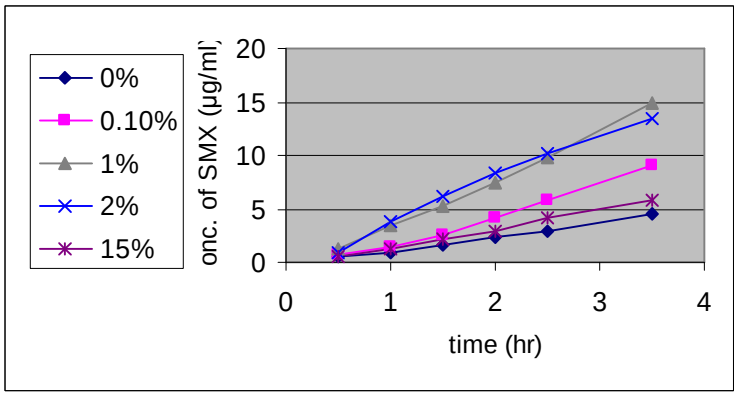

FIGURE 4. EFFECT OF DIFFERENT CONCENTRATIONS OF PEG 4000 ON THE ABSORPTION OF SMX FROM RABBIT ILEUM

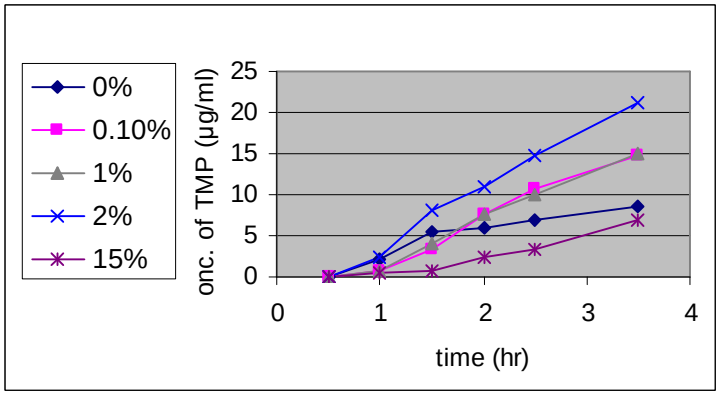

FIGURE 5. EFFECT OF DIFFERENT CONCENTRATIONS OF TWEEN 80 ON THE ABSORPTION OF TMP FROM RABBIT ILEUM

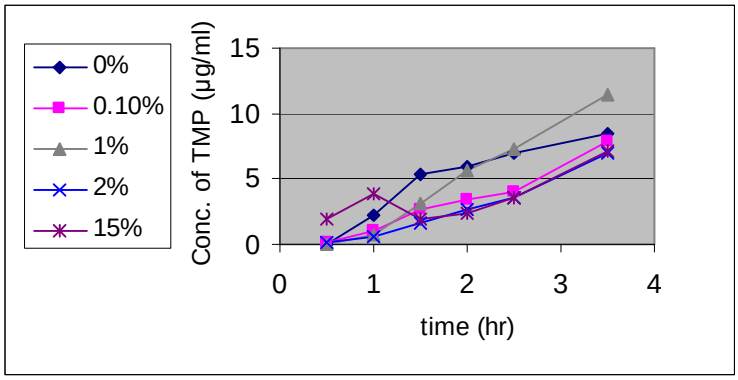

FIGURE 6. EFFECT OF DIFFERENT CONCENTRATIONS OF SLS ON THE ABSORPTION OF TMP FROM RABBIT ILEUM

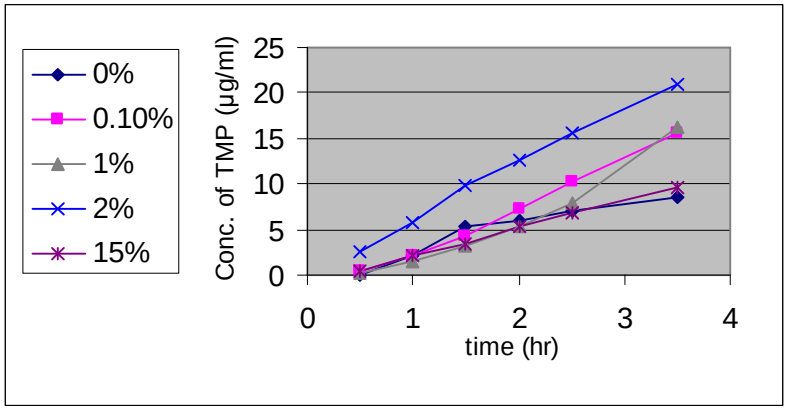

FIGURE 7. EFFECT OF DIFFERENT CONCENTRATIONS OF PEG 400 ON THE ABSORPTION OF TMP FROM RABBIT ILEUM

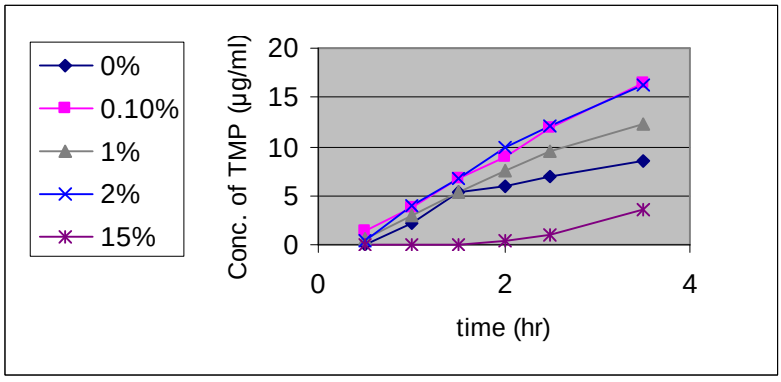

FIGURE .8 EFFECT OF DIFFERENT CONCENTRATIONS OF PEG 4000 ON THE ABSORPTION OF TMP FROM RABBIT ILEUM

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