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Competitive Binding of AD Agents to Scalp from Shampoo - A Novel Method for Evaluation

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Abstract

The present study describes a new method of evaluation of the competitive binding of the actives in AD shampoo. We have used Banana silk cloth and Velvet cloth to evaluate the extend of adsorption of actives from shampoo when shampoo contact with the cloth. Further spectrophotometric conformation of the actives in the residue in banana silk cloth and velvet cloth were also evaluated. Similarly, the microbial avoidance test was performed to test the therapeutic value of the actives adsorbed in the test material. Study findings clearly showed that our method is cost effective, rapid, less time consuming and reproducible for evaluating the anti-dandruff performance of shampoo. The above method also gives a comparable simulation model for scalp. Details are presented in the article.

Keywords: Dandruff; Shampoo; Climbazole; Zinc pyrithione

Introduction

Anti-dandruff (AD) shampoos are gaining importance with the increased incidence of dandruff at the global level. Newer and newer anti dandruff agents are getting developed but still a solution to the problem of dandruff remains far from near [1-3].

One of the key challenges in the management of dandruff is the delivery of AD agents onto the scalp from the shampoo system and the subsequent display of the activity. The shampoo systems in general will have very short contact time in the scalp and therefore a proper separation, release and deposition of the AD agents from the shampoo may not be occurring in most cases and that may be the reason most AD shampoo perform poorly despite having potent AD actives [4,5].

The need of the hour is not about having more potent anti-dandruff agents but how to ensure the proper release of the AD agents from shampoo during short contact time. The formulation homogeneity is extremely important and therefore the AD agent (s) must be miscible in the shampoo system and at the same time when the shampoo is diluted with water, the AD agents must get separated and binds to the scalp and should not get washed off with water [6].

To achieve the above purpose, rheology and judicious selection & use of other surfactants are important as well as the choice of conditioner and the wetting time of the shampoo.

We in the present paper discuss about a novel method to evaluate the competitive binding of AD agents from shampoo and the importance of formulation engineering to enhance the AD benefit. We have used velvet cloth and

banana silk cloth for the above purpose and the details are presented in the paper.

Materials and methods

Preparation of samples

1. Verdura Anti-dandruff shampoo- Proprietary formulation of Dr. JRK's Research and Pharmaceuticals Pvt. Ltd and is composed of dual AD actives such as Climbazole (CBZ) and Zinc pyrithrione (ZPTO) at 1 % each.
2. 27 % SLES was taken and was incorporated with 1% each of CBZ and ZPTO and dissolved well and then used. Its viscosity was measured to be 3,000 cps.
3. 27% SLES was taken and then its viscosity was increased to 30,000 cps with sodium chloride and then it was incorporated with 1% each of CBZ and ZPTO, dissolved well and then used.
4. A skeletal shampoo formulation was made with SLES, CAPB and water at 36, 5 and 59 % respectively. The above shampoo base was incorporated with 1% each of Climbazole and ZPTO and dissolved well and then used.

Determination of competitive deposition of AD agents

For the above purpose we have used velvet cloth as well as banana silk cloth.

The velvet cloth was used because it is woven with tufted fabric in which the cut threads are evenly distributed, and they are short, dense piles, likely to simulate the scalp with hair fibers.

Banana silk

The fabric is made with banana fiber along with synthetic silk and linen. The uniqueness of the fabric is that the banana fibers imbibe water and thereby likely to retain several residual actives even during short contact time.

Determination of diffusion time

The cloths, both velvet and banana silk were cut into small circular pieces of 1 inch radius. 1gm of the sample was placed at the center of the cloth and then the cloth was placed in a beaker containing water. The required support was given to the cloth from beneath to prevent the cloth getting immersed the water and the extent of diffusion of the sample into the water was observed and based on the observation, the time in minutes taken for the diffusion of the sample was noted down [7].

Spectrophotometric conformation of CBZ & ZPTO

1gm of the sample was placed at the center of the cloth and then the cloth was placed in a beaker containing water. The required support was given to the cloth from beneath to prevent the cloth getting immersed the water. After 5 minutes the cloth was removed, the remaining water was allowed to drain down and the residue was weighed, taken for both spectrophotometric and microbial avoidance study.

Similarly, the filtrate was used for the determination of CBZ and ZPTO by spectrophotometry. The wavelength length selected for CBZ was 278 & 283nm and wavelength selected for ZPTO was 247 & 322 nm [8,9].

Microbial avoidance study

The residual samples from the velvet and banana silk cloths were swabbed onto Sabroud's Dextrose Agar (SDA) plate, pre-inoculated with Candida albicans. The extent of growth of the organism around the swab marking was counted to ascertain the extent of deposition of AD actives and the resultant inhibition [10].

Preparation of Standard curve for Climbazole and Zinc pyrithione

The Climbazole and Zinc pyrithione at varying concentrations were prepared and read spectrophtometrically, a standard curve was prepared and was used for quantifying the amount of CBZ and ZPTO in the residue in velvet and banana silk cloths.

Quantification of CBZ and ZPTO in the residue and filtrate

The quantity of CBZ and ZPTO in the residue obtained with velvet cloth and banana silk cloth were calculated using the standard curve and a solution of CBZ and ZPTO were prepared in water adjusting to the above concentration equivalent to that of Verdura anti-scaling scalp shampoo residue and tested for microbial avoidance. Similarly the quantification of CBZ and ZPTO in the filtrate was also done using the standard graph of CBZ and ZPTO.

Results

The rate of diffusion of different samples was greater through velvet cloth than through banana silk. The diffusion of SLES was faster than SLES with higher viscosity or the skeletal shampoo base. Verdura anti-

scaling scalp shampoo exhibited almost even diffusion through both velvet cloth and banana silk (Table 1).

Sample details – (all samples contains 1% each of CBZ & ZPTO)	Diffusion of sample/ time in minutes	
	Velvet cloth	Banana silk
27 % SLES (3,000 Cps)	7	14
SLES (30,000 Cps)	11	18
Skeletal shampoo (35,000 Cps)	11	19
Verdura Anti-scaling scalp shampoo (40,000 Cps)	14	15

Table 1: Determination of diffusion time.

Spectrophotometric conformation of CBZ & ZPTO in the residue

The presence of relatively greater proportion of CBZ and ZPTO was observed in the residue of Verdura shampoo in both velvet and banana silk cloth. The presence of CBZ and ZPTO in the residue of other sample was minimal (Table 2).

Sample details – all contains 1% each of CBZ & ZPTO	Source of sample recovery/nm (Data shown in the table are absorbance value)							
	Velvet cloth				Banana silk			
	CBZ		ZPTO		CBZ		ZPTO	
	276	283	247	322	276	283	247	322
23 % SLES (3,000 Cps)	0.012	0.06	0.07	0.08	0.013	0.05	0.09	0.09
SLES (30,000 Cps)	0.001	0.01	0.05	0.03	0.002	0.006	0.004	0.008
Skeletal shampoo (35,000 Cps)	0.006	0.08	0.04	0.02	0.009	0.01	0.03	0.05
Verdura Anti-scaling scalp shampoo (40,000 Cps)	0.19	0.23	0.38	0.41	0.20	0.24	0.39	0.44

Table 2: Detection of the CBZ and ZPTO residues by UV Spectrophotometer.

Spectrophotometric conformation of CBZ & ZPTO in the filtrate

In the filtrate, the presence of CBZ and ZPTO was higher for all the samples except Verdura shampoo (Table 3).

The release of CBZ and ZPTO was low in the filtrate of Verdura anti-scaling scalp shampoo, indicating that CBZ and ZPTO may be getting adhered to the cloth.

Sample details – all contains 1% each of CBZ & ZPTO	Source of sample recovery/nm (Data shown in the table are absorbance value)							
	Velvet cloth				Banana silk			
	CBZ		ZPTO		CBZ		ZPTO	
	276	283	247	322	276	283	247	322
23 % SLES (3,000 Cps)	0.20	0.22	0.36	0.45	0.20	0.26	0.38	0.46
SLES (30,000 Cps)	0.21	0.18	0.33	0.43	0.22	0.28	0.30	0.42
Skeletal shampoo (35,000 Cps)	0.18	0.26	0.32	0.39	0.16	0.19	0.22	0.41
Verdura Anti-scaling scalp shampoo (40,000 Cps)	0.012	0.06	0.07	0.08	0.013	0.05	0.09	0.09

Table 3: The detection of the Climbazole and zinc pyrithione residues by UV Spectophotometer.

Microbial Avoidance Study

The residue was swabbed over SDA plate pre-inoculated with Candida albicans and none of the samples except Verdura anti-scaling scalp shampoo alone reduced the abundance of Candida albicans suggesting the strong presence of both CBZ and ZPTO in the residue of Verdura anti-scaling scalp shampoo (Table 4).

Sample details – all contains 1% each of CBZ & ZPTO	Measure of microbial growth	
	Velvet cloth	Banana silk
23 % SLES (3,000 Cps)	Abundant	Abundant
SLES (30,000 Cps)	Abundant	Abundant
Skeletal shampoo (35,000 Cps)	Abundant	Abundant
Verdura Anti-scaling scalp shampoo (40,000 Cps)	Minimal	Insignificant

Table 4: Abundant – luxuriant growth around the swabbed region, Minimal- small tiny countable colonies around the swabbed region, Insignificant – no to 1 or 2 colonies around the swabbed region.

Quantification of CBZ and ZPTO in the residue vis-à-vis microbial avoidance

The spectrophotometric confirmation of the residue of Verdura shampoo as well as other samples was made with the standard graph of CBZ and ZPTO. The findings reveal that the AD agents present in the residue of Verdura shampoo in velvet cloth was 80 mg and 60 mg respectively for ZPTO and CBZ. The quantity of ZPTO and CBZ in the residue obtained in banana silk was comparable with that of velvet cloth (Table 5). However the quantity of CBZ and ZPTO in the residue of other samples was quite insignificant.

On the contrary the quantity of CBZ and ZPTO in the filtrate was quite for other samples compared to Verdura shampoo (Table 6).

The anti- microbial activity of 80 mg and 60mg of ZPTO and CBZ respectively were tested by streaking the sample over SDA plate pre-inoculated with Candida albicans. The above concentrations of ZPTO and CBZ significantly reduced the growth of the organism.

Sample details – (all samples contains 1% each of CBZ & ZPTO)	Quantification in mg			
	Velvet cloth		Banana silk	
	CBZ	ZPTO	CBZ	ZPTO
27 % SLES (3,000 Cps)	7	13	8	9
SLES (30,000 Cps)	8	11	5	12
Skeletal shampoo (35,000 Cps)	5	10	6	13
Verdura Anti-scaling scalp shampoo (40,000 Cps)	60	80	50	82

Table 5: Quantification of CBZ and ZPTO in the residue.

Sample details – (all samples contains 1% each of CBZ & ZPTO)	Quantification in mg			
	Velvet cloth		Banana silk	
	CBZ	ZPTO	CBZ	ZPTO
27 % SLES (3,000 Cps)	60	75	39	88
SLES (30,000 Cps)	55	62	42	68
Skeletal shampoo (35,000 Cps)	46	73	56	77
Verdura Anti-scaling scalp shampoo (40,000 Cps)	8	15	9	4

Table 6: Quantification of CBZ and ZPTO in the filtrate.

Discussion

In the present study we have achieved near perfect simulation of scalp ecosystem by using velvet cloth and banana silk to evaluate the delivery mechanism of AD agents from shampoo. Although most sort after treatment product to contain dandruff is AD shampoo and hair cleansers but still more number of AD shampoos are getting launched in the market than any real relief for dandruff sufferers.

The development of newer AD agents has although shown great anti-dandruff activity in In vitro and also at clinical trial stage but still the formulations with such new AD agents have not given any great promise to the dandruff sufferers.

Why the proven clinical efficacy and invitro result obtained for a given AD agent in the laboratory is not getting translated in the real situation has raised more questions than answer. The above anomaly has led the

microbiologist and scientist to question whether dandruff is microbe led disease or a mere physiological phenomenon.

The profuse scalp scaling may occur due to microbial role and such situation also can be triggered by the allergic causes. In most cases of dandruff, abundant presence of the causative agent has been reported but the question is whether the abundance of the causative agent is responsible for the hyper-proliferation of the scalp cells or the hyper-proliferated scalp cells in turn increases the abundance of the causative agent in the scalp. Several studies have undoubtedly established *Pityrosporum ovale, Candida albicans* and *Staphylococcus epidermidis* to be commensal flora of human skin and scalp [11].

Unfortunately the above confusion in the etiology of dandruff has never raised any doubt about the delivery mechanism of AD agents from the shampoo as well as the competitive binding of AD agents on the scalp.

Shampoo is likely to have very short contact time with the scalp and therefore in the short contact time, the release and adherence of AD agents must happen. The formulation nuances and formulation intelligence is therefore extremely essential to achieve the above task [12].

If the formulation is made in such a way to ensure greater deposition of AD agents from shampoo, the treatment success may increase significantly and that may put the lid to the academic debate on whether the dandruff has microbial etiology or the proliferation of scalp cells facilitate microbial abundance.

The formulation intelligence in making AD shampoo has not happened so far may be due to the lack of a simple, cost effective, reliable, reproducible and relatable test model for evaluating the rate of release of AD agents from shampoo.

Over several years of screening and testing of many substances, we have identified velvet cloth and banana silk that can serve as best model for studying the competitive binding of AD agents from shampoo. Further the findings obtained with above method are also relatable with the scalp ecosystem.

Our present study has revealed that Verdura shampoo has taken slightly higher diffusion time when compared to other samples tested. In order to understand the presence of CBZ and ZPTO in the residue as well as in the filtrate of various samples in velvet cloth and banana silk, we have tested both the residue and the filtrate for the presence of CBZ and ZPTO by spectrophotometer and microbial avoidance test.

Interestingly the residue obtained from Verdura shampoo yielded higher quantity of CBZ and ZPTO when compared to other samples. Initially we presumed that Verdura shampoo may not be getting diffused through the velvet and banana silk. But our diffusion assay has clearly shown that the complete diffusion of the entire 1gm of shampoo has happened through both velvet cloth and banana silk in 15 minutes in time. We made attempts to quantify CBZ and ZPTO in the residue and the filtrate and found that the CBZ and ZPTO were found at higher levels in the residue of Verdura shampoo than the other samples tested.

Based on the percentage incorporation CBZ and ZPTO in Verdura shampoo vis-à-vis a gram of shampoo used for test, we are supposed to extract 100mg of CBZ and ZPTO from all the samples tested. In the case of verdure shampoo we have obtained 60% and 80% of CBZ and ZPTO respectively in the residue. CBZ and ZPTO were found to be higher in the filtrate of all other samples except Verdura shampoo. This clearly suggests that the greater separation and greater deposition of CBZ and ZPTO is being facilitated more effectively by Verdura shampoo formulation intelligence than the other formulations.

The above finding when we try to extrapolate to the scalp condition above 50% of the AD actives from Verdura shampoo is likely to bind to the scalp and thereby great relief to dandruff also can be expected.

We have prepared fresh solution of CBZ and ZPTO in water by adjusting the concentration of CBZ and ZPTO in the residue of Verdura shampoo and all the samples were tested for microbial avoidance. The concentration of CBZ and ZPTO in the residue of Verdura shampoo as well as the fresh solution of CBZ and ZPTO inhibited the growth of Candida albicans more or less equally. This suggests that CBZ and ZPTO from Verdura shampoo that remained in the residue are therapeutically effective as well. Whereas, in other formulations, the diffusion of CBZ and ZPTO was higher in the filtrate suggesting that the base along with AD actives may get washed off easily during hair wash.

Our finding clearly states the importance of formulation engineering of AD shampoo and the associated scientific intelligence. The above important dimension of AD shampoo we could unravel mainly due to our capability in identifying and establishing velvet cloth

and banana silk model and its closer simulation to the scalp condition.

In Verdura shampoo homogeneity of the formulation has been achieved but at the same time the AD actives have been immobilized in the shampoo system therefore the AD actives are evenly dispersed but not in the dissolved state. This technique we presume may be the reason why Verdura has shown superior release of CBZ and ZPTO. All the four formulations/samples tested contain 1% each of CBZ and ZPTO. However Verdura shampoo alone could release above 50% of the actives in short contact time.

Our present study clearly warrants the need for rigorous look at the formulation engineering in order to enhance the efficacy of the active agents. The active agents may be effective but if the formulation base does not allow the spontaneous release of the actives, under such situations, the formulation may not be effective.

The role of base denaturing/ inactivating the AD actives such as CBZ and ZPTO cannot be ignored. Therefore we presume that Verdura shampoo is the most suitable shampoo formulation for CBZ and ZPTO. Formulations of the therapeutically effective preparations must focus both on the active agents as well as formulation intelligence. Further a suitable model also is required to validate the efficacy which we have achieved in the present study.

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