International Journal of Biomedical and Clinical Sciences
Vol. 1, No. 2, 2016, pp. 36-42
http://www.aiscience.org/journal/ijbcs

Compatibility Evaluation of Salivae Miltiorrhizae Liguspyragine Hydrochloride and Glucose Injection Based on Isothermal Titration Calorimetry

Jian Ke[1], Yong Luo[2], Xian-Peng Ma[3], Chang-Qiong Bi[3], Wei Wei[3], Jian-Hao Pan[1], Jing Zhao[4], Fa-Huan Ge[5], Hong Nie[1, *]

[1]Guangdong Province Key Laboratory of Pharmacodynamics Constituents of TCM and New Drugs Research, College of Pharmacy, Jinan University, Guangzhou, China
[2]Cell Biology Department, College of Life Science and Technology, Jinan University, Guangzhou, China
[3]Guizhou Jingfeng Injection Co. Ltd., Guiyang, China
[4]State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China
[5]School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou, China

Abstract

This article is to evaluate safety risk of Salivae Miltiorrhizae Liguspyragine Hydrochloride and Glucose Injection (SGI) with six injections, which are used in combination with SGI in clinical practice, by isothermal titration calorimetry (ITC). The six injections are as follows: Levofloxacin Hydrochloride and Sodium Chloride Injection (LSI), Cefoperazone Sodium and Sulbactam Sodium for Injection (CSI), Alprostadil Injection (AI), Dextran 40 Glucose Injection (DI), Ossotide Injection (OI) and Glycerol Fructose and Sodium Chloride Injection (GSI). SGI was titrated by six injections and the thermodynamical parameters of each titration were obtained by ITC. In addition, changing information in appearances, pH values and insoluble particles of each samples during 4 hours after mixed at room temperature (25°C) were determined respectively. Among the six titration reactions, five of their Gibbs free energy values are negative (LSI, CSI, AI, OI and GSI); one of its Gibbs free energy value is positive (DI). Appearances and pH values of six injections mixed with SGI showed no obvious change during 4 hours at ambient temperature. And the total number of insoluble particles in the admixture of the two injections was exceeded upper limit standard of Chinese Pharmacopoeia (Ch.P.). In general, combined with appearances, pH values and insoluble particles results, this research indicated that using SGI in combination with the five injections (i.e. LSI, CSI, AI, OI and GSI) in clinical practice should pay attention to the ADR because Gibbs free energy values of the five titrations are negative, which means spontaneous reaction took place when they are mixed. While SCI suits to be combination with DI because Gibbs free energy value of the titration is positive, which means there is no energy changes and no reaction occurred.

Keywords

Compatibility Evaluation, Isothermal Titration Calorimetry, Salivae Miltiorrhizae Liguspyragine Hydrochloride, Glucose Injection

Received: September 16, 2016 / Accepted: September 25, 2016 / Published online: October 9, 2016

* Corresponding author
E-mail address: tnieh@jnu.edu.cn (Hong Nie)

1. Introduction

SGI is a chemical injection, which is originated from *Salvia miltiorrhiza Bunge.* and *Ligusticum chuanxiong Hort.* (100ml: equivalent to 20mg tanshinol and 100mg ligustrazine hydrochloride), and was approved on market by the CFDA in 2003. SGI is widely used in treatment of the occlusion of cerebrovascular disease and ischemic vascular diseases [1]. Sometimes, SGI is used in combination with other injections in order to improve efficacy in clinical practice. However, some adverse drug reactions (ADR) are reported for its combination with other injections (like LSI, CSI and AI). Six injections were selected to mix with SGI in order to simulate the combination process of the injections *in vitro* incording to the ADR cases reported from practices since they using combined with each other normally. They are Levofloxacin Hydrochloride and Sodium Chloride Injection (LSI), Cefoperazone Sodium and Sulbactam Sodium for Injection (CSI), Alprostadil Injection (AI), Dextran 40 Glucose Injection (DI), Ossotide Injection (OI) and Glycerol Fructose and Sodium Chloride Injection (GSI).

Traditionally, methods for evaluating combination safety of injectable drugs are mainly by visual observation, insoluble particle assay [2, 3], pH assay [4, 5], osmolality assay [5, 6], HPLC assay [6-10]. These methods could be used to detect changing information of admixture, but have some problems such as inconvenient operation and low sensitivity. ITC is a micro-thermal method, taking the total concentration of the reagents and the change of the heat of reaction as a function, and obtaining the thermodynamic parameters based on a certain mathematical model. Then the type of the titration reaction can be further judged according to the relationship between the thermodynamic parameters [11, 12]. ITC can be used to monitor the fast response, and it is mainly used in the interaction between macromolecules and/or small molecules [13], the effects of environment on the reaction and the research of enzyme kinetics [14, 15]. But it is still fresh to evaluate safety risk of injection combination, FENG Xue applied ITC to evaluate compatibility of Yiqifumai Injection with Vitamin C Injection and 5% Glucose Injection [16]. YAN Dan applied ITC to evaluate the interaction in Qingkailing Injection with Ceftizoxime Sodium Injection and 5% Glucose Injection [17]. And their work confirms that this application of ITC is feasible. ITC is a real-time and high sensitivity method, which can rapidly determine the change of physical and chemical characteristics of drug combination.

The appearance, pH value and insoluble particle of the admixtures were measured as supplementary evidences to see if the ITC results are parallel.

2. Experimental

2.1. Materials

Salivae Miltiorrhizae Liguspyragine Hydrochloride and Glucose Injection (SGI; Guizhou Jingfeng Injection Co. Ltd.; Approval number: H52020703; Name of commodity: Bai Sai Tong); Levofloxacin Hydrochloride and Sodium Chloride Injection (LSI; Daiichi Sankyo (Beijing) Co. Ltd.; Approval number: H20010782); Cefoperazone sodium and Sulbactam Sodium for injection (CSI; Hainan General Sanyo Pharmaceutical Co. Ltd.; Approval number: H20030932); Alprostadil Injection (AI; Harbin Pharmaceutical Group Biological Engineering Co. Ltd.; Approval number: H20084565); Dextran 40 Glucose Injection (DI; Sichuan Kelun pharmaceutical Co. Ltd.; Approval number: H51020230); Ossotide Injection (OI; Anhui Hongye Pharmaceutical Co. Ltd.; Approval number: H20053157); Glycerol Fructose and Sodium Chloride Injection (GSI; Chen Ou Pharmaceutical Co. Ltd.; Approval number: H20057114); Glucose Injection (GI; Sichuan Kelun pharmaceutical Co. Ltd.; Approval number: H51020634).

2.2. Main Apparatus

Isothermal titration calorimetry (General Electric Company, model: MicroCal ITC200); pH meter (Mettler Toledo-United States instruments Shanghai Co. Ltd, model: FE20); Intelligent particle detector (Tianjin Tianhe Instrument Co. Ltd., model: GWF-8JA); Osmolality tester (YASN International Group Co. Ltd., model: OM815)

Preparation of samples

SGI solution was prepared by a bottle of Salivae Miltiorrhizae Liguspyragine Hydrochloride and Glucose Injection (100ml: equivalent to 20mg salviae miltiorrhizae and 100mg ligustrazine hydrochloride); LSI solution was prepared by a bottle of Levofloxacin Hydrochloride and Sodium Chloride Injection (100ml: 0.3g); CSI solution was prepared in a manner like this: Took a bottle of Cefoperazone sodium and Sulbactam Sodium for injection (1g), then dissolved it with 5ml of 5% glucose injection. After Transferred to a 250ml volumetric flask, it was diluted with 5% glucose injection to the scale line; AI solution was prepared by an Alprostadil Injection (2ml:10g) and dissolved in 10ml 5% Glucose Injection; DI solution was prepared by a Dextran 40 Glucose Injection (500ml:25g); OI solution was prepared in a manner like this: took an Ossotide Injection (5ml:25mg) and precisely shifted it to a 100 ml volumetric flask, and it was diluted with 5% glucose injection to the scale; GSI solution was prepared by a bottle of Glycerol Fructose Sodium Chloride Injection (250ml: 25g, fructose 12.5g, sodium chloride 2.25g).

2.3. Methods

2.3.1. Isothermal Titration Calorimetry

300μl combined injection was titrated by 80μl SGI, respectively. At constant temperature of 25°C, stirring rate was controlled as 1000 rpm, after the instrument is automatically balanced 60s, then started titrating. One titration process costs 20 drops, each drop equals 0.5μl. Each drop takes 1s and the interval time is 200s. Data acquisition and analyses are done by MicroCal-enabled Origin software in the process of titration.

Each titration was accompanied by a change of heat, then reached equilibrium. MicroCal-enabled Origin software can be used to analyze the experimental data automatically. Once concentrations of samples are input, the software will automatically conduct the reactivity spectrum analysis. After data fitting, two thermodynamic parameters of the mixture between two injection solutions are calculated, namely reaction enthalpy change ΔH and equilibrium constant *K*. According to the thermodynamic parameter relation formulas, (1) and (2), Gibbs free energy ΔG and entropy change ΔS can be obtained:

$$\Delta G=-RT\ln K \quad (1)$$

$$\Delta G=\Delta H-T\Delta S \quad (2)$$

In the formula above, R represents the gas constant and T represents the absolute temperature. Formula (2) showed that the ΔG is composed of ΔH and −TΔS, and the type of reaction is determined by how much those two parts contribute to the Gibbs free energy. If $|\Delta H|>T|\Delta S|$, the reaction is enthalpy driven reaction, namely chemical reaction; If $|\Delta H|<T|\Delta S|$, the reaction is entropy driven reaction, namely physical reaction [11].

2.3.2. Appearance and pH Assay

The SGI solution and six combined solutions were respectively taken 5ml into clean beakers. At room temperature (25°C), pH value of every sample was determined, and its appearance was observed and recorded. Then the six combined solutions above was added with equal volume of SGI solution respectively, the pH value of each sample was determined at 0, 0.5, 1, 2 and 4h after mixed respectively, and the changes of their appearance were observed and recorded. Judgmental standards of appearance are as follows: I°: the mixture is clear, transparent, no sediment and other abnormal substances; II°: the mixture is turbid, but no flocculent material produced; III°: the mixture is turbid, and there are granular material; IV°: the mixture is gelatinous, also has serious flocculent material.

2.3.3. Insoluble Particle Assay

In accordance with the insoluble particle determination method of Chinese Pharmacopoeia (Ch.P.) 2010 Edition, SGI solution and six combined solutions 40ml were respectively taken in clean beakers, which were washed twice with ultrapure water. At room temperature (25°C), insoluble particle number of every sample was determined. Then the six combined solutions above were added with equal volume of SGI solution respectively, the insoluble particle number of each sample was determined at 0, 0.5, 1, 2 and 4h after mixed respectively.

3. Results

3.1. Isothermal Titration Calorimetry

While recording the change of heat generated by each titration, the reacting process spectrum of two kinds of injection was obtained. The spectrum of heat change in the continuous titration process is obtained by integrating each titration peak. The reacting process spectrum and spectrum of heat change are collectively known as the spectrum of reacting activity (Figure 1).

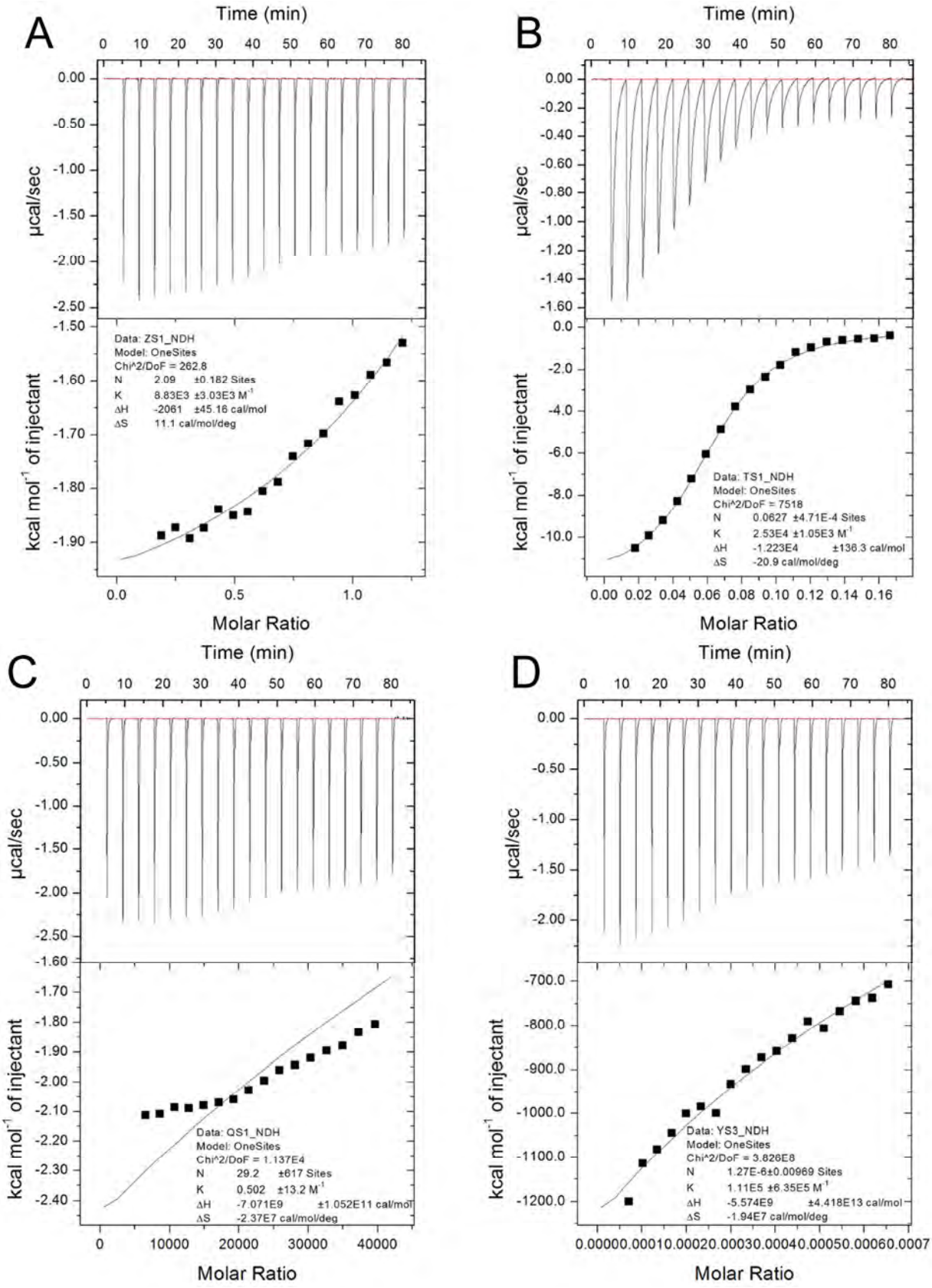
Table 1. Thermodynamic parameters of solvation between different injections.

Titrated samples	ΔH (cal/mol)	ΔS (cal/mol/deg)	TΔS (cal/mol)	ΔG (cal/mol)	Major type of reactions
LSI	-2.06×10^{3}	1.11×10^{1}	3.31×10^{3}	-5.37×10^{3}	Physical[a]
CSI	-1.22×10^{4}	-2.09×10^{1}	-6.23×10^{3}	-6.00×10^{3}	Chemical[a]
AI	-7.07×10^{9}	-2.37×10^{7}	-7.07×10^{9}	-5.00×10^{6}	Chemical[a]
DI	-5.77×10^{9}	-1.94×10^{7}	-5.78×10^{9}	1.00×10^{7}	Non-spontaneous
OI	-2.93×10^{3}	1.05×10^{1}	3.13×10^{3}	-6.06×10^{3}	Physical[a]
GSI	-1.47×10^{3}	1.52×10^{1}	4.53×10^{3}	-6.00×10^{3}	Physical[a]

[a] The reaction type is spontaneous reaction.

As shown in the Table 1, ΔG of reactions between SGI with LSI, CSI, AI, OI, GSI were negative, which can identify that spontaneous reaction took place in solvation between the five combined injections and SGI. It can be directly deduced that chemical change took place in solvation between SGI and CTI or AI ($|\Delta H|>T|\Delta S|$), which suggests that there might be a chemical bond formation or rupture in the titration process; On the other hand, physical change took place in solvation between SGI and LSI, OI, GSI ($|\Delta H|<T|\Delta S|$), which suggested that the physical properties of some substances in

the process of titration could be changed. In addition, ΔG of the reaction between SGI with LSI was positive, which means the reaction of DI and SGI is a non-spontaneous one. The solvation system was quite stable, for neither chemical reaction nor physical reaction had been seen in the solvation between DI and SGI.



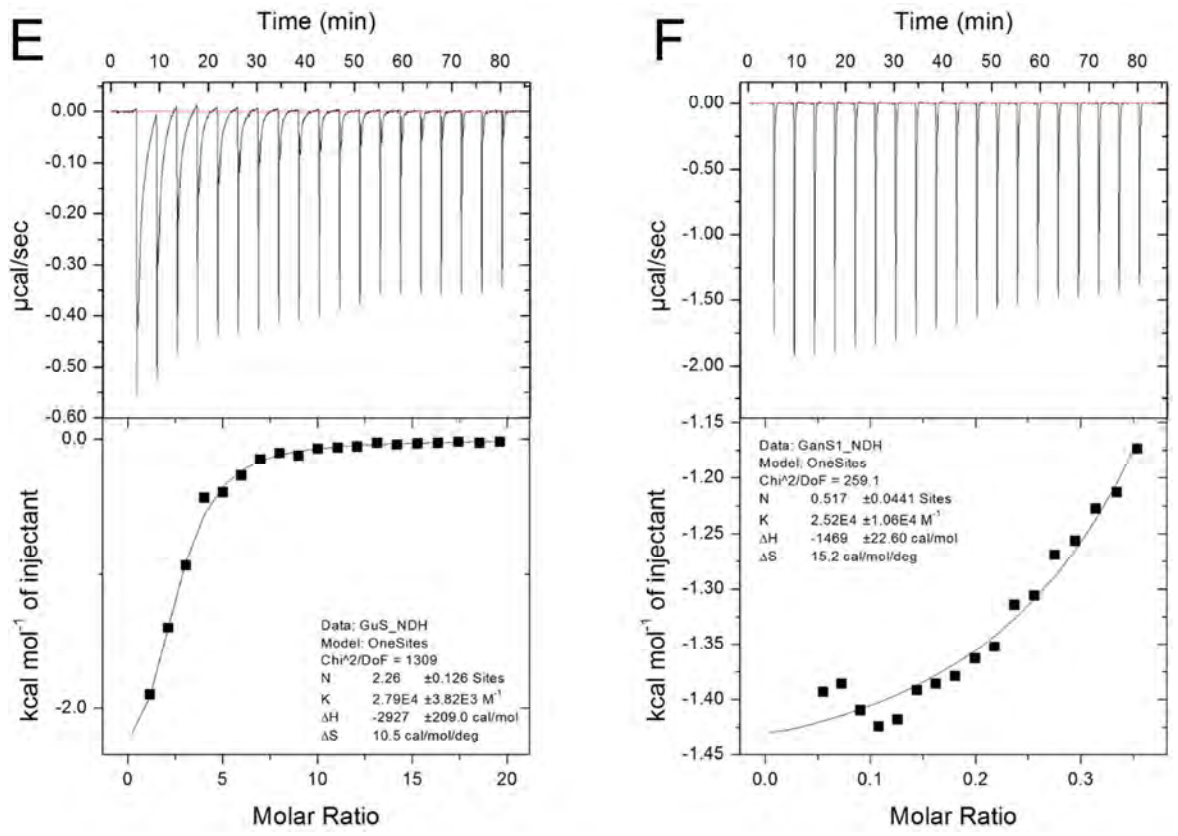


Figure 1. The spectrums of reacting activity of SGI solution and different solution. 25°C, stirring rate 1000 rpm, interval time 200s. (A) LSI; (B) CSI; (C) AI; (D) DI; (E) OI; (F) GSI.

3.2. Appearance, pH Value and Osmolality

Within 4 hours, there was no significant change in the appearance and pH values of mixtures between SGI and any combined injection. (Table 2)

Table 2. Determination of appearance, pH values and osmolality of six mixed solutions within 4 hours (25°C).

		Time (h)					
		Before mixed	**0**	**0.5**	**1**	**2**	**4**
LSI	Appearance	I°	I°	I°	I°	I°	I°
	pH	4.82	4.69	4.69	4.66	4.67	4.68
CSI	Appearance	I°	I°	I°	I°	I°	I°
	pH	4.53	4.60	4.65	4.65	4.66	4.72
AI	Appearance	II°	II°	II°	II°	II°	II°
	pH	5.88	4.73	4.72	4.67	4.70	4.73
DI	Appearance	I°	I°	I°	I°	I°	I°
	pH	4.13	4.54	4.59	4.60	4.72	4.70
OI	Appearance	I°	I°	I°	I°	I°	I°
	pH	5.72	4.75	4.75	4.75	4.77	4.81
GSI	Appearance	I°	I°	I°	I°	I°	I°
	pH	3.89	4.50	4.51	4.55	4.57	4.60

Note: I°: the mixture is clear, transparent, no sediment and other abnormal substances; II°: the mixture is turbid, but no flocculent material produced; III°: The mixture is turbid, and there are granular material; IV°: the mixture is gelatinous, also has serious flocculent material.

3.3. Insoluble Particles

After addition of SGI, the total number of insoluble particles (including the larger than 10μm ones and larger than 25μm ones) in LSI and CSI both increased significantly within 4 hours. And the total number of insoluble particles in the admixture of LSI and SGI exceeded upper limit standard of Ch.P. [18]. After addition of SGI, there is a clear downward trend in AI's total number of insoluble particles within 4 hours, suggesting that SGI might has an effect of solubilization on AI. It is worth noting that CSI and AI are concentrated solutions for injection (Table 3).

Table 3. Determination of insoluble particles in six mixed solutions within 4 hours (25°C).

	particle size	Time (h)					
		Before mixed	0	0.5	1	2	4
LSI	≥25μm	0	0.8	0.6	1.9	4.5	4.6
	≥10μm	0.6	13.8	18.0	61.1	128.2	144.1
CSI	≥25μm	6.3	3.2	4.3	7.4	6.1	8.5
	≥10μm	59.7	34.2	45.5	88.8	90.5	123.5
AI	≥25μm	0.4	0.2	0.4	0.7	0.3	0.1
	≥10μm	279.4	113.8	99.4	98.5	87.7	82.3
DI	≥25μm	0.6	0.8	0.8	0.9	1.6	1.0
	≥10μm	1.0	8.7	14.6	22.2	24.2	22
OI	≥25μm	1.1	0.4	0.7	0.6	0.8	0.9
	≥10μm	15.1	9.9	13.1	11.4	11.8	12.3
GSI	≥25μm	0.2	0.5	0.6	1.0	0.5	0.8
	≥10μm	15.2	14.8	18.8	20.2	19.3	22.6

4. Discussions

Mixture of two injections *in vitro* is not equal to the combination of the injections in clinic, but ITC is currently a better method to simulate injection combination and reveal their energy change of the mixing process and hint the reactions between injections. Reactions mean properties change, which may lead to uncertainty in admixtures. That uncertainty means potential safety risk.

To some extent, test results of pH assay and insoluble particle assay were in agreement with ITC results. But, ITC has more advantages than the traditional methods. For example, ITC can obtain more information, consume less sample and has the characteristics of high sensitivity, simple operation, little interference, good reproducibility. It is worth mentioning that, in our previous study, we conducted HPLC assay, one method that is widely used in the evaluation of combination risk. However, HPLC results showed no apparent abnormality when chromatographs of SGI, combined injections and their admixtures were put together to make a comparison. Therefore, those traditional evaluation methods are difficult to get relatively comprehensive information, and ITC may become a new method that can be widely spread in combination risk evaluation of injections.

5. Conclusions

In summary, combined with appearances, pH values and insoluble particles results, this research indicated that attention should be paid to ADRs when using SGI in combination with the five injections (i.e. LSI, CSI, AI, OI and GSI) in clinical practice because Gibbs free energy values of the five titrations are negative, which means spontaneous reaction took place when they are mixed. The chemical or physical changes in the admixtures suggest that there is potential safety risk.. And SCI suits to be combination with DI because Gibbs free energy value of the titration is positive, which means there is no energy changes and no reaction occurred.

Besides, we mainly discussed the risk assessment of the compatibility of injection *in vitro*, and provided the experimental evidence for the clinical safety. However, the related mechanism should be further studied.

Acknowledgements

The authors gratefully acknowledge the financial supports by National Natural Science Foundation of China (81673634).

References

[1] Qi XF, He J. (2007). Salvia miltiorrhiza ligustrazine hydrochloride and glucose injection in the treatment of acute cerebral infarction. Neural Injury and Functional Reconstruction 2: 212.

[2] Shen GP. (2009). The change of insoluble particle before and after qingkailing injection compatibility with four kinds of antibiotics. Chin J Clin Ration Drug Use 2: 43-45.

[3] Pan L. (2008). Detection of insoluble particles in 6 traditional Chinese medicine injections. China Pharm 19: 56-58.

[4] Xiao XJ, Liu HY. (2008). Studies on stabilities of Qingkailing injections after used together with transfusions and antibiotics. Med Pharm Yunnan 29: 64-65.

[5] Xiong QJ, Zhu XS, Zheng F. (2009). Studies on Danhong injections and 5 kinds of transfusions. China Pharm 12: 167-168.

[6] Zhang YM, Yan D, Zhang P, et al. (2010). Quality control of Shuanghuanglian freeze-dried powder for injection based on its HPLC-ELSD fingerprints and biological profiles. Acta Pharm Sin 45: 93-97.

[7] Zhu SM, Kuang R, Wang D, et al. (2010). Investigation of osmolality and hemolysis ratio in Shenmai injection. Drug Eval Res 33: 11-14.

[8] Lu J, Li SF, Ma BZ, et al. (2008). Pansporin and 3 kinds of transfusions. Chin J Hosp Pharm 28: 76-78.

[9] Hu CQ, Xu MZ, Ma Y, et al. (2008). Determination of the allergic impurities in the parenteral injection of Chinese traditional medicines containing Salvia miltiorrhiza. Acta Pharm Sin 43: 518-522.

[10] Chen XX. (2011). Study on the compatible stability of shenmai injection with fructose injection by HPLC and UV. Strait Pharmaceutical Journal 23: 27-28.

[11] Li YS, Xia XX, Li XH, et al. (2007). Application of microcalormetric in the science of life. Chem Ind Times 21: 64-67.

[12] Hiroyasu O, Adrian VC, Dong X, et al. (2002). Strongest and most specific HIV-1 protease inhibitors have both strong enthalpy and entropy binding components. Protein Sci 11: 1908-1916.

[13] Gulden CU, Nicola LBP. (2010). Thermodynamics of binding interactions between divalent copper and chitin fragments by isothermal titration calorimetry. Carbohydr Polym 81: 8-13.

[14] Xu H, Zhu QQ, Lu J, et al. (2010). Studies on thermodynamic nature of stereoselectivity for ruthenium (II) polyptridyl complex binding to DNA. Inorg Chem Commun 13: 711-714.

[15] Maximova K, Trylska J. (2015). Kinetics of trypsin-catalyzed hydrolysis determined by isothermal titration calorimetry. Anal Biochem 486: 24-34.

[16] Feng X, Yan D, Y an Y, et al. (2011). Compatibility evaluation of Chinese medicines injections based on isothermal titration calorimetry. Acta Pharmaceutica Sinica 46 (3): 322-328.

[17] Yan D, Chen LH, Feng X, et al. (2012). Characterization of interaction in Qingkailing Injection with clinic administration based on isothermal titration calorimetry. Chin Tradit Herb Drugs 43: 2217-2221.

[18] National Pharmacopoeia Commission. (2010). Pharmacopoeia of the People's Republic of China, Volume II. 9th ed. Bejing: China Medical Science and Technology Press Appendix: 71-73.