



Effect of Acid-Base Property in Conjunction with Molecular Descriptors for Chemical Drugs and Bioactive Ingredients of Traditional Chinese Medicine on Prediction of Oral Absorption by Bipartitioning Micellar Chromatography

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This research was aimed to predict per cent of human oral absorption (HOA %) for drugs by bipartitioning micellar chromatography (BMC). A data set of 102 chemical drugs containing acidic, basic, neutral and amphiprotic drugs from various structure classes with oral absorption rate data available was studied to construct prediction models of oral absorption for all chemical drugs, acidic chemical drugs, basic chemical drugs and neutral chemical drugs respectively and proved the effect of the acid-base property of drugs on oral absorption. Oral absorption was reciprocally correlated to the negative value of the capacity factor (-1/k). The correlation was improved with the addition of molecular descriptors. According to established prediction models for chemical drugs, bipartitioning micellar chromatography was applied to predict oral absorption of the organic acids and alkaloids of traditional Chinese medicines (TCM) for bioactive ingredients basing on constructed quantitative structure-activity relationship/quantitative retention-activity relationship models of chemical drugs. The results showed that application was predictable and practical in bioactive ingredients of traditional Chinese medicines.

Key Words: Bipartitioning micellar chromatography, Oral absorption, Traditional Chinese medicines.

INTRODUCTION

Most commercially available drugs are administered orally as this noninvasive method is the preferred route of drug administration. Thus, understanding which properties need to be optimized in order to enhance oral absorption is quite basic. The passive transcellular permeability of a compound that is crucial for its oral absorption is often modeled on lipophilicity, molecular size and/or hydrogen bonding¹. Studies have unambiguously demonstrated that lipophilicity is a key parameter in predicting and interpreting permeability. Lipophilicity commonly expressed by the octanol/water partition coefficient as log P, has been successfully used to predict passive drug absorption *in vivo* for series of homologous compounds². But it fails to encode some important recognition forces, such as ionic bonds, which are notably important because charged forms of some molecules are able to partition into phospholipids bilayer³. For this reason, we must take the ionization of drugs under physiological environment into account according to their acid-base property. Lu *et al.*⁴ amended linear solvation energy relationship (LSER) by the introduction of a molecular electronic factor to establish quantitative structure-retention

relationship of bipartitioning micellar chromatography (BMC) system. It was found that when the mean net charge per molecule (*d*) was introduced into LSER as the sixth variable, the LSER regression coefficients and predictive capability were significantly improved⁴. Moreover, the lipophilicity itself is inadequate to estimate solute's ability to penetrate a membrane barrier. Several studies have also shown that physico-chemical descriptors of molecules such as lipophilicity, polar surface area (PSA) and hydrogen bonding descriptors correlate with cell culture permeability and intestinal absorption^{5,6}. Num_H_Donors_Lipinski (NHDL) is the number of hydrogen bond donors (a donor is any O-H or N-H group), yielding a poor permeability to biomembrane if large than 5, according to Lipinski's 'rule of 5'⁷. Molecular polar surface area has been shown to correlate very well with the human intestinal absorption, Caco-2 monolayer permeability^{8,9}. But the polar surface area has been demonstrated a non-linear relationship with permeability, which declines sigmoidally as polar surface area increases¹⁰.

Chromatographic models are universally used to study passive absorption due to experimental simplicity, low cost, accuracy and high throughput, among which the immobilized

artificial membrane (IAM), immobilized-liposome chromatography (ILC) and biopartitioning micellar chromatography (BMC) system are well recognized models. Biopartitioning micellar chromatography is a chromatographic modality optimized in order to describe the biological behaviour of drugs which usually is comprised of a C₁₈ reversed stationary phase and polyoxyethylene (23) lauryl ether (Brij35) micellar mobile phases in adequate experimental conditions provides a partitioning environment that can be used as a system that mimics drug biopartitioning. It has been testified to be useful to study human oral absorption prediction, chemical toxicity, skin permeability and penetration of drugs across the blood-brain barrier (BBB)¹¹⁻¹⁶. Moreover, biological activity of different oral drugs were studied by our research group¹⁷⁻²².

Traditional Chinese medicines (TCM) makes great contributions for the prosperity and proliferation of people as their successful treatment and prevent for various diseases. They are usually very complex mixtures containing up to hundreds or even thousands of different ingredients and thus differ greatly from synthetic drugs in some aspects. So screening and analysis of bioactive ingredients of traditional Chinese medicines are very important not only for quality control of the crude drugs but also for elucidating the principles of therapeutic action of traditional Chinese medicines²³. Usually, traditional methods for screening bioactive ingredients of traditional Chinese medicines are mostly animals' models. The whole-animal model can, apparently, reveal the efficiency, side effects and toxicity of medicines, but it is time-consuming, arduous and inappropriate for direct screening bioactive ingredients of traditional Chinese medicines. However, traditional Chinese medicines have similar retention behaviour on biopartitioning micellar chromatography column for their physico-chemical properties and absorption mechanisms are analogous with chemical drugs. Therefore, bioactive properties of traditional Chinese medicines can be predicted by biopartitioning micellar chromatography for its simplicity, low cost, accuracy and high throughput.

But when biopartitioning micellar chromatography is used to predict oral absorption of bioactive ingredients of traditional Chinese medicines, the retention may not be obtained accurately because of interference of other ineffective and unknown components. Therefore, in this paper, parts of monomers of traditional Chinese medicines for bioactive ingredients with available literatures about their oral absorption condition were just preliminarily predicted. Firstly, we constructed prediction models of chemical drugs for oral absorption, which were introduced to retention factor (*k*) and molecular structure descriptors (describe molecular structure mathematically) of selected chemical drugs according to their acid-base property. And then, we predicted oral absorption using biopartitioning micellar chromatography basing on previously mature studies. Finally, prediction oral absorption of monomers of traditional Chinese medicines for bioactive ingredients by models of chemical drugs and comparison predicted value with literatures reported value of their oral absorption were conducted.

EXPERIMENTAL

The mobile phase consisted of 0.04 mol/L polyoxyethylene (23) lauryl ether (Brij35, Acros, NJ, USA) with 0.01 mol/L

sodium dihydrogenphosphate (analytical-reagent grade, Kelong, Chengdu, China) and was adjusted to pH 7.4 which is the plasmatic pH value by sodium hydroxide. In order to reproduce the osmotic pressure of biological fluids, Sodium chloride (9.20 g/L, analytical-reagent grade, Kelong, Chengdu, China) was added to the micellar mobile phase. The sodium chloride concentration was close to physiological concentration of biological fluids. Water was from a millipore (Billerica, MA, USA) synergyTM 185 system and was degassed before HPLC. The mobile phases injected into the chromatograph were filtered through 0.45 μm micro porous membrane.

Among the 102 medicine drugs, piroxicam, isoniazid, verapamil, caffeine, meloxicam, hydrochlorothiazide, bumetanide, furosemide, pindolol were used as control articles provided by analysis test center of West China School of Pharmacy and the others were crude drug or formulations which would not affect their retention behaviour in this study because of the dilution of the mobile phase, donated by the pharmaceutical and pharmaceutical chemistry laboratories of West China School of Pharmacy, Sichuan University (Chengdu, China). Acidic reference substances of traditional Chinese medicines including chlorogenic acid, ferulic acid, caffeic acid, cinnamic acid, rhein, salvianolic acid B, tanshinol and basic reference substances of traditional Chinese medicines including barbering, colchicine, hyaconitine, theopylline, sinomenine, colchicines, tetrahydropalmatine which were purchased from National Institute for the control of Pharmaceutical and Biological Products.

Water-soluble drugs were dissolved in mobile phase solution. Lipophilic drugs were first dissolved in methanol (analytical-reagent grade, Kelong, Chengdu, China) and then were diluted with water to get appropriate concentration. Stock standard solutions of reference substance and crude drug of analytes were prepared by dissolving 10 mg of the compound in 10 mL volumetric flask. Working solutions were prepared by dilution of the stock standard ones using the Brij35 solution. For those pharmaceutical preparations of analytes, working solutions were prepared by dissolving 10 mg of the tablet or capsule powders of the drugs in 10 mL volumetric flask, then centrifuged at 1000 × 10 rpm for 5 min. The working solutions injected into the chromatograph were filtered through 0.45 μm microporous membranes (Xinya, Shanghai, China), respectively. All the solutions were stored under refrigeration at 4 °C before analysis.

The retention of drugs was measured using an LC-6A chromatograph with an LC-6A pump, an SPD-6AV UV-visible detector and a CTO-6A column thermostat (Shimadzu, Japan). Data were collected and processed on a Compaq computer installed with HP-Chemstation software (A0402, 1996). The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA), with a 20 μL loop. The HPLC column was a Kromasil C₁₈ column (5 μm, 150 × 4.6 mm i.d.) with a phenomenex security GuardTM C₁₈ guard cartridge. The mobile phase flow rate was 1.0 mL/min. The UV detection of chemical drugs was monitored at 220, 254, 270 and 300 nm and active ingredients of traditional Chinese medicines were set at 270, 280 and 240.

All the assays were carried out at 37 °C for simulating human body temperature. The retention data in biopartitioning micellar chromatography were calculated as capacity factors, $k = (t_r - t_0)/t_0$, where t_r is the retention time of the test compound and t_0 is the column dead time, which is the first fluctuation of baseline, determined by injecting water. The k values used in this study were the average value of triplicate. The retention data were highly reproducible.

Statistical analysis: The data set was analyzed using Microsoft® Excel 2003 (Microsoft Corporation). Multiple linear regression was used to carry out on the data set using SPSS 12.0 software (the SPSS for windows version 12.0, SPSS Inc. Chicago, USA). Stepwise regression analysis which is one of the MLR methods was used to determine the most significant descriptors. Molecular descriptors were calculated by Discovery Studio 2.5 (Accelrys Software Inc, San Diego, CA, USA). ChemDraw® Ultra 8.0.3 (Cambridge soft corporation, USA) was used. For each regression, the following requirements of significant regression analysis were observed: n , R^2 , SE, F and p , in which n is the number of points used in the regression, R^2 is the square of the overall correlation coefficient, SE is the standard deviation and F is Fischer's F-statistic, which are used to control fit ability and statistics significance of regression mode. T test is used to prove that partial regression coefficient before each variable is meaningful or not in multiple linear regression (MLR) equation. Variation inflation factor (VIF) determined the multicollinearities among the descriptors. Variation inflation factor was calculated for each descriptor in the model as $1/(1-r^2)$. Multicollinearities were considered to exist when the variation inflation factor was greater than 10 and the model was considered to reconstruct. In addition, root mean squared error of calibration (RMSEC) was estimated predictive ability of the models.

RESULTS AND DISCUSSION

Retention/structure -oral absorption relationships of chemical drugs: For most drugs, the predominant mechanism of oral absorption is *via* the transcellular route. It relies on the ability of the molecule to partition into and move across gastrointestinal epithelium membranes. The most important features of a drug that influence this partitioning are solubility, permeability and molecular size, polarity and lipophilicity. A study suggests that 62.9 % of drugs are ionizable, of which 14.5 % are acids, 67.6 % are bases and 17.9 % are ampholytes of various types. Many drugs contain one or more ionizable groups and their lipophilicity is pH dependent. These biologically active molecules are at least partially ionized at biological pH values and absorption could be influenced by the membrane penetration of the neutral form and the receptor binding of the ionized form.

The first step in current study was to calculate bulk properties and molecular descriptors of the selected chemical drugs which were passively absorbed in intestinal wall, including neutral, acidic, basic and amphiprotic drugs with oral absorption % data available²³⁻²⁸ and to measure the retention (capacity factor k) of each drug on the biopartitioning micellar chromatography column as an indicator for the drug partitioning into cell membrane. The next step was to correlate calculated

descriptors and experimentally measured capacity factor against oral absorption %. Since an equation containing an excessive number of independent variables can be too cumbersome to use and is likely to be over parameterized, we utilized stepwise regression to refine the model and to select most important descriptors to generate regression equation of all chemical drugs, acidic chemical drugs, basic chemical drugs and natural chemical drugs, respectively. The findings of the study and their implications are elaborated in the subsequent sections.

All chemical drugs: The sample set of 102 chemical drugs with different acidity was random divided into training set including 84 drugs which was used to construct model and test set including 18 drugs which was used to evaluate predictability of model. The specific drugs, molecular descriptors, experimental oral absorption and predicted oral absorption calculated by equation (1) were showed in the Table-1 (supplementary files). The MLR equation was as follows:

$$\text{Oral absorption \%} = 114.163 (\pm 4.63) - 5.469 (\pm 1.867) 1/k_{\text{BMC}} - 4.584 (\pm 1.004) \text{Num_H_Donors_Lipinski} - 1.8151 (\pm 0.0558) \text{Kappa_2} - 8.727 (\pm 2.757) \text{Num_Terminal Rotomers} \quad (1)$$

Ionizable drugs in the given data set are partly or fully in ionized form under experimental conditions. The retention behaviour of the neutral and ionized forms was quite different on the biopartitioning micellar chromatography column because an electrostatic force is involved in the interactions between the ionized form and the phospholipids membrane, but it was not the case with neutral compounds. As can be observed, the regression coefficient of equation (1) ($R^2 = 0.746$) was a little poor and SE was slightly large, for which the following were listed as possible reasons: (1) Chemical drugs selected for constructing model of all chemical drugs are structurally diverse. The acidic chemical drugs and base chemical drugs may ionized partially at physiological pH and the retention behaviours on the biopartitioning micellar chromatography of neutral and ionized forms were quite different because electrostatic forces may be involved in the interaction between the ionized form and modified stationary phase of biopartitioning micellar chromatography, which might contribute to the poor relevance. (2) The oral absorption % values -biopartitioning micellar chromatography model has some limitations that only relate the retention behaviour and molecular structure feature to the oral absorption % values but it is physically unrealistic. It is unable to take the chemical and bacterial degradation of the drugs at the absorption site and the first-pass metabolism in the intestinal cells into consideration¹¹. From the magnitude of coefficients indicated that Num_Terminal Rotomers and $1/k$ were the two most important molecular descriptors determining oral absorption % in the biopartitioning micellar chromatography system studied. The log k (at pH 7.0 or 7.4) is often considered as effective molecular descriptor, indicative of the potential absorption properties of drugs. The retention (capacity factor k) on the biopartitioning micellar chromatography column is as an indicator for the drug partitioning into cell membrane. NTR are constitutional descriptors. Terminal rotamer is defined as either a non-terminal sp^3 atom connected to three-terminal atoms of the same type, or a non-terminal sp^2 atom connected to two-terminal atoms of the same type. The molecular descriptor k contributes the oral absorption % of the

TABLE-1
 MOLECULAR DESCRIPTORS, EXPERIMENTAL AND PREDICTED OA % AND RESIDUAL VALUES (EXPERIMENTAL OA % - PREDICTED ORAL ABSORPTION %) OF ALL CHEMICAL DRUGS, ACIDIC CHEMICAL DRUGS, BASIC CHEMICAL DRUGS AND NATURAL CHEMICAL DRUGS

Compound	1/k _{BMC}	OA _{Exp} (%)	NHDL	Kappa ₂	NTR	OA _{Pre1} (%)	RV ₁	K3A	JY	NAR	NSB	NH	OA _{Pre2} (%)	RV ₂	IC	MS	NR5	PSA	OA _{Pre3} (%)	RV ₃	OA _{Pre4} (%)	RV ₄		
Training set																								
1	Isoniazid	1.72	20	7	13.98	0	29.84	-9.84	1.53	2.89	1	0	0	16.14	3.86	-	-	-	-	-	-	-	-	
2	Amitriptyline	0.01	95	0	7.51	0	100.11	-5.11	3.09	2.07	2	1	1	96.38	-1.38	-	-	-	-	-	-	-	-	-
3	Ondansetron	0.1	100	0	6.14	0	101.25	-1.25	1.94	1.78	3	0	0	99.56	0.44	-	-	-	-	-	-	-	-	-
4	Aminophenazoe	0.14	100	0	5.33	0	102.13	-2.13	1.75	2.43	1	1	1	96.45	3.55	-	-	-	-	-	-	-	-	-
5	Oxazepam	0.04	97	2	6.41	0	92.58	4.42	2.47	2.25	2	1	1	96.25	0.75	-	-	-	-	-	-	-	-	-
6	Aprobarbital	0.09	85	2	4.89	0	94.62	-9.62	2.95	3.18	0	1	1	91.82	-6.82	-	-	-	-	-	-	-	-	-
7	Phenylbutazone	0.1	90	0	8.39	0	97.09	-7.09	2.84	2.04	2	0	0	85	5.00	-	-	-	-	-	-	-	-	-
8	Benazepril	0.12	37	5	12.89	2	48.25	-11.25	6.23	1.45	2	0	0	38.47	-1.47	-	-	-	-	-	-	-	-	-
9	Phenobarbital	0.08	100	2	5.33	0	93.96	6.04	1.6	2.56	1	0	0	94.55	5.45	-	-	-	-	-	-	-	-	-
10	Phenytoin	0.05	98	2	5.78	0	93.59	4.41	1.69	2.25	2	0	0	99.22	-1.22	-	-	-	-	-	-	-	-	-
11	Piroxicam	0.12	99	2	7.09	0	90.09	8.91	2.47	2.11	2	1	1	89.85	9.15	-	-	-	-	-	-	-	-	-
12	Probenecid	0.13	90	1	7.7	1	84.62	5.38	3.86	3.08	1	0	0	88.41	1.59	-	-	-	-	-	-	-	-	-
13	Ibuprofen	0.08	95	1	5.92	1	88.69	6.31	3.48	2.77	1	0	0	85.29	9.71	-	-	-	-	-	-	-	-	-
14	Diazepam	0.03	100	0	6.41	0	101.83	-1.83	2.36	2.24	2	1	1	99.46	0.54	-	-	-	-	-	-	-	-	-
15	Felodipine	0.06	100	1	9.27	0	91.53	8.47	3.8	2.76	1	2	2	95.35	4.65	-	-	-	-	-	-	-	-	-
16	Flurbiprofen	0.19	95	1	6.44	1	86.03	8.97	2.55	2.44	2	0	0	89.02	5.98	-	-	-	-	-	-	-	-	-
17	Flumazenil	0.11	95	0	6.86	0	99.76	-4.76	2.33	2.23	2	0	0	90.29	4.71	-	-	-	-	-	-	-	-	-
18	Furosemide	0.14	67	4	7.05	1	72.03	-5.03	3.61	2.22	2	0	0	71.74	-4.74	-	-	-	-	-	-	-	-	-
19	Flunitrazepam	0.04	100	0	7.49	1	90.95	9.05	2.62	2.3	2	1	1	96.94	3.06	-	-	-	-	-	-	-	-	-
20	Hexobarbital	0.05	95	1	4.94	0	99.70	-4.7	1.7	2.48	0	2	2	95.07	-0.07	-	-	-	-	-	-	-	-	-
21	Warfarin sodium	0.11	93	1	7.92	0	93.25	-0.25	2.99	2.17	2	1	1	89.04	3.96	-	-	-	-	-	-	-	-	-
22	Ciprofloxacin	0.11	69	2	6.96	1	81.76	-12.76	2.73	1.82	1	1	1	70.43	-1.43	-	-	-	-	-	-	-	-	-
23	Methylprednisolone	0.06	82	3	6.5	0	87.56	-5.56	2.28	1.79	0	2	2	80.57	1.43	-	-	-	-	-	-	-	-	-
24	Trimethoprim	0.17	97	4	8.02	0	78.50	18.5	3.07	2.53	2	0	0	91.3	5.70	-	-	-	-	-	-	-	-	-
25	Lamotrigine	0.08	98	4	5.1	0	85.29	12.71	2.03	2.65	2	0	0	101.11	-3.11	-	-	-	-	-	-	-	-	-
26	Lorazepam	0.05	93	2	6.63	0	92.02	0.98	2.65	2.28	2	1	1	93.75	-0.75	-	-	-	-	-	-	-	-	-
27	Clobazam	0.04	87	0	6.63	0	101.27	-14.27	2.38	2.26	2	0	0	93.32	-6.32	-	-	-	-	-	-	-	-	-
28	Chlordiazepoxide	0.03	100	1	6.84	0	96.49	3.51	2.61	2.2	2	2	2	102.41	-2.41	-	-	-	-	-	-	-	-	-
29	Lormetazepam	0.04	98	1	6.86	0	96.30	1.700	2.64	2.32	2	1	1	97.3	0.7	-	-	-	-	-	-	-	-	-
30	Losartan	0.1	80	2	11.23	0	82.73	-2.73	4.3	1.63	4	0	0	85.92	-5.92	-	-	-	-	-	-	-	-	-
31	Clonazepam	0.05	98	1	7.27	1	86.66	11.34	2.77	2.25	2	1	1	91.56	6.44	-	-	-	-	-	-	-	-	-
32	Meloxicam	0.09	90	2	6.72	0	91.24	-1.24	2.52	2.1	2	1	1	90.56	-0.56	-	-	-	-	-	-	-	-	-
33	Midazolam	0.02	100	0	6.72	0	101.41	-1.41	2.56	2.04	3	1	1	105.07	-5.07	-	-	-	-	-	-	-	-	-

34	Minoxidil	0.92	28	4	12.68	2	40.81	-12.81	2.26	2.04	1	0	0	31.69	-3.69	-	-	-	-	-	-	-	-
35	Naproxen	0.14	99	1	5.76	1	88.06	10.94	2.35	2.5	2	0	0	96.35	2.65	-	-	-	-	-	-	-	-
36	Nitrendipine	0.07	88	1	9.97	1	81.35	6.65	3.96	2.74	1	2	2	93.38	-5.38	-	-	-	-	-	-	-	-
37	Pravastatin	0.33	34	4	11.92	3	42.62	-8.62	7.33	2.17	0	2	2	29.29	4.71	-	-	-	-	-	-	-	-
38	Amrinone	0.24	93	3	4.68	0	88.14	4.86	-	-	-	-	-	-	-	3.33	-1.94	0	68	90.52	2.48	-	-
39	AmLodipine	0.05	80	3	12	0	77.53	2.47	-	-	-	-	-	-	-	4	-4.6	0	100	80.93	-0.9	-	-
40	Olanzapine	0.21	91	1	6.48	0	94.37	-3.37	-	-	-	-	-	-	-	3.73	-3.67	1	59	89.88	1.12	-	-
41	Omeprazole	0.05	80	1	8.13	0	93.79	-13.79	-	-	-	-	-	-	-	3.96	-4.91	1	98	85.22	-5.2	-	-
42	Alprenolol	0.05	93	2	8.99	0	87.65	5.35	-	-	-	-	-	-	-	3.31	-3.72	0	41	93.48	-0.5	-	-
43	Atenolol	1.96	56	4	9.03	0	48.94	7.06	-	-	-	-	-	-	-	3.22	-2.86	0	85	58.67	-2.7	-	-
44	Allopurinol	0.85	90	2	2.56	0	87.18	2.82	-	-	-	-	-	-	-	2.65	-0.17	1	75	92.4	-2.4	-	-
45	Prilocaine	0.12	90	2	7.35	0	89.61	0.39	-	-	-	-	-	-	-	3.75	-3.46	0	41	88.88	1.12	-	-
46	Imipramine	0.02	95	0	7.51	0	99.95	-4.95	-	-	-	-	-	-	-	3.15	-4.52	0	6.5	97.19	-2.2	-	-
47	Bupivacaine	0.03	80	1	8.59	0	93.25	-13.25	-	-	-	-	-	-	-	3.99	-5.71	0	39	84.91	-4.9	-	-
48	Scopolamine	0.38	95	1	6.14	0	92.37	2.63	-	-	-	-	-	-	-	3.57	-1.45	1	62	91.6	3.4	-	-
49	Irbesartan	0.06	92	1	10.73	0	88.83	3.17	-	-	-	-	-	-	-	3.58	-6.84	3	87	94.85	-2.9	-	-
50	Irbesartan	2.78	34	5	3.92	2	23.71	10.29	-	-	-	-	-	-	-	2.73	1.59	0	131	30.94	3.06	-	-
51	Fluphenazine	1.79	65	1	9.67	0	64.03	0.97	-	-	-	-	-	-	-	3.43	-4.13	0	55	60.35	4.65	-	-
52	Haloperidol	0.02	95	1	9.47	0	91.78	3.22	-	-	-	-	-	-	-	3.08	-5.7	0	41	92.91	2.09	-	-
53	Labetalol	0.07	90	5	10.22	0	71.42	18.58	-	-	-	-	-	-	-	3.19	-4.74	0	96	87.78	2.22	-	-
54	Ranitidine	1.41	50	2	10.68	1	54.76	-4.76	-	-	-	-	-	-	-	3.79	-3.9	1	128	51.7	-1.7	-	-
55	Ribavirin	3.57	33	5	5.33	0	26.33	6.67	-	-	-	-	-	-	-	3.74	-0.25	2	144	33.78	-0.8	-	-
56	Metoprolol	0.33	90	2	9.83	0	81.77	8.23	-	-	-	-	-	-	-	3.12	-3.43	0	51	89.92	0.08	-	-
57	Nadolol	1.69	40	4	7.71	1	46.83	-6.83	-	-	-	-	-	-	-	3.98	-3.19	0	129	41.9	-1.9	-	-
58	Norfloxacin	0.11	71	2	7.49	1	80.77	-9.77	-	-	-	-	-	-	-	3.98	-2.44	0	76	75.22	-4.2	-	-
59	Procaine	0.26	85	3	8.16	0	81.39	3.61	-	-	-	-	-	-	-	3.38	-2.43	0	58	89.81	-4.8	-	-
60	Propranolol	0.23	90	2	7.7	0	87.26	2.74	-	-	-	-	-	-	-	3.25	-4.16	0	41	90.17	-0.2	-	-
61	Zidovudine	0.45	100	2	6.64	0	85.82	14.18	-	-	-	-	-	-	-	3.38	-0.3	1	20	97.6	2.4	-	-
62	Nortriptyline	0.03	95	1	7.32	0	95.6	-0.6	-	-	-	-	-	-	-	3.22	-6.27	0	12	93.11	1.89	-	-
63	Tramadol	0.14	90	1	6.64	0	95.16	-5.16	-	-	-	-	-	-	-	3.93	-3.34	0	33	87.91	2.09	-	-
64	Timolol	0.33	90	2	8.02	1	76.39	13.61	-	-	-	-	-	-	-	3.17	-0.7	1	101	83.32	6.68	-	-
65	Sulpiride	0.67	86	3	8.39	0	74.62	11.38	-	-	-	-	-	-	-	3.27	-2.69	1	110	82.98	3.02	-	-
66	Terbutaline	0.65	73	4	5.56	1	66.90	6.1	-	-	-	-	-	-	-	2.85	-2.1	0	73	76.49	-3.5	-	-
67	Verapami	0.02	90	0	14.53	0	86.96	3.04	-	-	-	-	-	-	-	3.35	-7.51	0	64	85.6	4.4	-	-
68	Pentobarbital	0.02	100	2	5.56	0	94.47	5.53	-	-	-	-	-	-	-	3.33	-2.53	0	35	96.28	3.72	-	-
69	Urapidil	0.2	78	1	10.86	0	86.42	-8.42	-	-	-	-	-	-	-	3.99	-4.1	0	68	81.99	-4	-	-
70	Cimetidine	0.33	84	3	9	0	78.76	5.24	-	-	-	-	-	-	-	3.57	-2.92	1	114	85.54	-1.5	-	-
71	Bromperidol	0.02	95	1	9.47	0	91.78	3.22	-	-	-	-	-	-	-	3.08	-6.12	0	41	92.24	2.76	-	-

72	Pindolol	0.29	87	3	6.96	0	83.15	3.85	-	-	-	-	-	-	3.57	-3.01	1	57	91.1	-4.1	-	-
73	Amobarbital	0.03	95	2	5.56	0	94.31	0.69	-	-	-	-	-	-	3.33	-2.44	0	45	95.37	-0.4	-	-
74	Aciclovir	2.56	30	4	5.56	0	46.08	-16.08	-	-	-	-	-	-	4.55	-	-	-	-	-	-	-
75	Theophylline	0.63	89	1	3.29	0	93.78	-4.78	-	-	-	-	-	-	2.085	-	-	-	-	-	-	-
76	Digoxin	0.43	81	1	8.79	1	77.97	3.03	-	-	-	-	-	-	-	-	-	-	-	84.14	-	-
77	Dexamethasone	0.04	92	3	6.5	0	87.87	4.13	-	-	-	-	-	-	3.893	-	-	-	-	-	-	-
78	Mannitol	4.76	16	3	5.61	0	16.50	-0.5	-	-	-	-	-	-	1.892	-	-	-	-	-	-	-
79	Testosterone	0.02	100	1	4.75	0	100.51	-0.51	-	-	-	-	-	-	3.74	-	-	-	-	-	-	-
80	Caffeine	0.47	92	0	3.54	0	100.34	-8.34	-	-	-	-	-	-	2.836	-	-	-	-	-	-	-
81	Prazosin	0.07	100	2	9.43	0	86.53	13.47	-	-	-	-	-	-	3.023	-	-	-	-	-	-	-
82	Corticosterone	0.03	100	2	6.27	0	93.00	7	-	-	-	-	-	-	3.569	-	-	-	-	-	-	-
83	Primidone	0.14	90	2	5.10	0	93.46	-3.46	-	-	-	-	-	-	3.603	-	-	-	-	-	-	-
84	Hydrocortisone	0.21	89	3	6.25	0	85.70	3.3	-	-	-	-	-	-	3.779	-	-	-	-	-	-	-
85	Quinine	0.07	93	1	7.32	0	94.98	-1.98	-	-	-	-	-	-	4.085	-	-	-	-	-	-	-

Test set

1	Alprazolam	0.04	95	0	8.481	0	97.86	-2.86	2.96	2.03	3	1	1	99.3	-4.3	-	-	-	-	-	-	-
2	Phenazone	0.31	97	0	4.245	0	101.56	-4.56	1.4	2.57	1	1	1	91.34	5.66	-	-	-	-	-	-	-
3	Bumetanide	0.1	100	1	3.196	1	93.36	6.64	5.1	3.51	2	0	0	102.53	-2.53	-	-	-	-	-	-	-
4	Propylthiouracil ethyl	0.3	76	1	12.133	0	82.45	-6.45	2.01	2.63	0	1	1	71.74	4.26	-	-	-	-	-	-	-
5	Fluvastatin	0.08	100	1	3.228	1	93.6	6.4	5.04	2.39	3	1	1	96.14	3.86	-	-	-	-	-	-	-
6	Carbamazepine	0.07	100	2	5.551	0	93.64	6.36	1.7	2.12	2	1	1	100.08	-0.08	-	-	-	-	-	-	-
7	Hydrochlorothiazide	0.1	90	4	4.281	0	86.5	3.5	2.24	2.79	1	0	0	89.76	0.24	-	-	-	-	-	-	-
8	Valsartan	0.28	55	5	13.185	1	53.88	1.12	5.66	1.79	3	0	0	58.24	-3.24	-	-	-	-	-	-	-
9	Lamivudine	1.28	87	2	2.888	0	79.89	7.11	1.04	3.64	0	2	2	80.68	6.32	-	-	-	-	-	-	-
10	Amoxapine	0.03	95	1	6.857	0	96.46	-1.46	-	-	-	-	-	-	3.61	-4.15	0	37	90.97	4.03	-	-
11	Orphenadrine	0.02	95	0	8.444	0	98.22	-3.22	-	-	-	-	-	-	3.54	-4.78	0	12	92.85	2.15	-	-
12	Atropine	0.24	98	1	7.051	0	92.92	5.08	-	-	-	-	-	-	3.59	-2.47	1	50	93.44	4.56	-	-
13	Paracetamol	0.26	80	3	5.133	0	86.98	-6.98	-	-	-	-	-	-	3.99	-3.98	0	59	81.91	-1.9	-	-
14	Lidocaine	0.08	90	1	7.438	0	94.56	-4.56	-	-	-	-	-	-	3.70	-3.2	0	32	91.16	-1.2	-	-
15	Venlafaxine	0.23	97	1	7.320	0	92.43	4.57	-	-	-	-	-	-	3.00	-3.88	0	33	93.49	3.51	-	-
16	Oxprenolol	0.13	97	2	6.834	0	90.35	6.65	-	-	-	-	-	-	3.06	-3.23	0	51	94.21	2.79	-	-
17	Chlorpromazine	0.01	95	0	7.951	0	99.25	-4.25	-	-	-	-	-	-	3.34	-4.85	0	32	92.96	2.04	-	-

$1/k_{BMC}$: reciprocal of capacity factors k on BMC column; $OA_{EX}(\%)$: Experimental percent of oral absorption; $OA_{PRE1}(\%)$: Predicted percent of oral absorption of all chemical drugs; NHDL: Num_H_Donors_Lipinski; NTR: Num_Terminal Rotomers; RV_1 : Residual value of all chemical drugs; Molecular descriptors of all chemical drugs are $1/k_{BMC}$, NHDL, Kappa_2 and NTR. Training set of all chemical drugs in Table1 are from 1 to 85 and test set of all chemical drugs are from 1 to 17; K3A: Kappa_3_AM; NAR:Num_Aromatic_Rings; NSB: Num_StereoBonds; NH: Num_Hydrogens; $OA_{PRE2}(\%)$: Predicted percent of oral absorption of acidic chemical drug; RV_2 : Residual value of acidic chemical drug; Molecular descriptors of acidic chemical drugs are $1/k_{BMC}$, KA3, JY, NAR, NSB and NH. Training set of acidic of chemical drugs in Table-1 are from 1 to 37 and test set of acidic of chemical drugs are from 1 to 9; NTR: Num_Terminal Rotomers; NR5:Num_Rings 5; MS: Molecular_Soublity; PSA: Polar Surface Area; $OA_{PRE3}(\%)$: Predicted percent of oral absorption of basic chemical drugs; RV_3 : Residual value of basic chemical drugs; Molecular descriptors of basic chemical drugs are $1/k_{BMC}$, NTR, IC, MS, NR5 and PSA. Training set of basic chemical drugs in Table1 are from 38 to 73 and test set are from 10 to 17; $OA_{PRE4}(\%)$: Predicted percent of oral absorption of natural chemical drugs; RV_4 : Residual value of natural chemical drugs; Molecular descriptors of natural chemical drugs are $1/k_{BMC}$ and IC. Training set of natural chemical drugs in Table1 are from 74 to 85.

compounds and the molecular descriptor NTR will reduce the oral absorption %.

Acidic chemical drugs: A sample set including 46 diverse structural acidic chemical drugs was random divided into training set with 37 acidic chemical drugs and test set with 9. The specific drugs, molecular descriptors, experimental oral absorption % and predicted oral absorption % calculated by equation (2) were shown in the Table-1 (supplementary files). The MLR equation was as follows:

$$\text{Oral absorption \%} = 18.362 (\pm 19.763) - 1.416 (\pm 4.500) 1/k_{\text{BMC}} - 10.986 (\pm 1.743) \text{KAPPA_3_AM} + 28.975 (\pm 5.640) \text{JY} + 13.095 (\pm 2.614) \text{Num_Aromatic_Rings} + 5.977 (\pm 2.062) \text{Num_StereoBonds} + 0.884 (\pm 0.412) \text{Num_Hydrogens}$$

$$N = 37; R^2 = 0.906; SE = 7.26; F = 48.13; P < 0.001 \quad (2)$$

Acidic chemical drugs mostly contain carboxylic group (-COOH), the other does not have such a group. Lu *et al.*⁴ found that the dissociation of the carboxylic group had a great impact on the retention of the acidic drugs with such a group on biopartitioning micellar chromatography. Its dissociation decreased the retention of acidic drugs because of strong polarity and weak fat-soluble of molecule in the dissociation state leading to difficulty transmembrane diffusion⁴. Besides, such categories of clonazepam and lorazepam, they are without carboxylic group in their structures indeed had long experimental retention. This seemed to show strong interactions between this ionizable molecule and the biopartitioning micellar chromatography column. Experimental errors and spatial matching might contribute to this strong retention. The regression coefficient increased from 0.746 to 0.928, suggesting the consideration of acid-base property is necessary. In addition, the coefficient of KAPPA_3_AM (K3A) and JY are larger than other molecular descriptors in magnitudes suggesting that K3A and JY impacting most on the oral absorption % of acid drugs. K3A is α -modified shape index of order three. The α -modified Kappa shape indices are refinements of the kappa shape indices that take into the contribution of covalent radii and hybridization states consideration, reflecting the shape characteristic of molecule as well. JY is one of Balaban Indices belonging to topological descriptors, describes connective and branch information of atoms composed of in molecules and impacts the steric and hydrophobic function of molecule. The negative coefficient of K3A suggests that molecular shape can not allowed good absorption. And JY is positive to absorption.

Basic chemical drugs: A sample set including 44 diverse structural basic chemical drugs is random divided into training set with 36 basic chemical drugs and test set with 8. The specific drugs, molecular descriptors, experimental oral absorption and predicted oral absorption obtained from flowing equation (3) with 6 parameters by means of MLR method were showed in Table-1 (supplementary files).

$$\text{Oral absorption \%} = 132.737 (\pm 9.808) - 17.368 (\pm 1.554) 1/k_{\text{BMC}} - 10.420 (\pm 2.611) \text{Num_Terminal Rotomers} - 8.694 (\pm 2.987) \text{IC} + 1.603 (\pm 0.562) \text{Molecular_Soublity} + 4.305 (\pm 1.513) \text{Num_Rings} - 0.088 (\pm 0.042) \text{Polar Surface Area}$$

$$N = 36; R^2 = 0.929; SE = 5.27; F = 63.10; P < 0.001 \quad (3)$$

The pKa values of the basic drugs considered the range of 7-10.2. An ionizable compound can be assumed to exist in

its total ionized form when pH value is and less than one unit away from pKa (pKa -1 for base). The retention of basic chemical drugs does not seem to be significantly affected by ionization; attractive interactions play an important role in the retention. Kotecha *et al.*¹ performed an immobilized artificial membrane chromatographic method on the retention behaviours of 28 drugs represented acidic, basic, neutral and amphoteric drugs for predicting human oral absorption. The authors observed that basic drugs (pKa > 8) that remain almost ionized (80 to 100 %) at pH 7.4 showed lipophilicity based retention regardless of the degree of ionization. In another paper, Barbato *et al.*²⁷ also concluded experimentally that even in the ionized form, the retention of basic drugs was as strong as if they were uncharged. From the magnitudes of coefficients, descriptors of $1/k_{\text{BMC}}$ and NTR also impact mostly on oral absorption.

Neutral chemical drugs: This category consisted of 12 neutral chemical drugs with diverse structures and physico-chemical characteristics. Inclusion criteria were used in the selection of the model drugs. These included the following: (1) neutral drugs without ionization at physiological condition; and (2) these drugs should be of oral delivery and the absorbed fractions in humans were available. So this model was developed using a relatively small training set. The value of oral absorption % of 12 kinds of neutral chemical drugs and corresponding molecular structure descriptors were seen in Table-1 (supplementary files).

Multiple linear regression was applied to construct optimal oral absorption % prediction model of neutral chemical compound containing two independent variables.

$$\text{Oral absorption \%} = 138.347 (\pm 18.963) - 21.680 (\pm 1.914) 1/k_{\text{BMC}} - 11.290 (\pm 4.983) \text{IC}$$

$$N = 12; R^2 = 0.955; SE = 6.54; F = 94.778; P < 0.001 \quad (4)$$

Since Brij35 is non-ionic surfactant, natural chemical drugs can be allowed more retained than basic and acidic chemical drugs. As a result they can obtain good oral absorption consequence of easy transcellular diffusion.

As can be observed, the *p*-values obtained for four models (eqns. 1-4) indicated that the relationships between these parameters and the oral absorption % were statistically significant at the 95 % confidence level. The coefficients obtained for those models were also significant at the same confidence. When MLR was used to construct model each variable should perform t test. From the Table-2 (supplementary files) listed, t value of each variable was large than the standard value of t for four models (all chemical drugs, $t\alpha/2 = 1.99$; acidic chemical drugs, $t\alpha/2 = 2.042$; basic chemical drugs, $t\alpha/2 = 2.045$; natural chemical drugs, $t\alpha/2 = 2.262$) at 95 % confidence level, which indicated that each variable affected significantly to the oral absorption %. Meanwhile, various inflation factor (VIF) calculated for each variable in the four models was less than 5, suggesting that the model was robust because multicollinearities among variables were not existing. Figs. 1c-3c and Fig. 4b (supplementary files) show the corresponding residual plots (Experimental oral absorption % - Predicted oral absorption %). There was a random distribution of the residuals and practically they were all statistically small which suggest, from a qualitative point of view, the adequacy of the

TABLE-2
VALUE OF t AND VARIATION INFLATION FACTOR (VIF) OF EACH VARIABLE FOR EQNS. 1-4

Variables in each equation		VIF	t
All chemical compounds	$1/k_{BMC}$	1.615	-8.285
	Num_H_Donors_Lipinki	1.572	-4.530
	Kappa_2	1.051	-3.318
	Num_Terminal Rotomers	1.030	-0.322
Acidic chemical compounds	$1/k_{BMC}$	1.269	-11.426
	KAPPA_3_AM	2.931	-6.308
	JY	2.761	5.138
	Num_Aromatic_Rings	3.426	5.010
	Num_Stereo Bonds	1.592	2.898
	Num_Hydrogens Hydrogens	4.717	2.145
Basic chemical compounds	$1/k_{BMC}$	2.130	-11.174
	Num_Terminal Rotomers	1.873	-3.990
	IC	1.367	-2.911
	Molecular_Solubility -Solubility	1.477	2.850
	Num_Rings 5	1.379	2.846
	Polar surface area	2.779	-2.090
Natural chemical compounds	$1/k_{BMC}$	1.940	-11.328
	IC	1.940	-2.266

model to data. But some factors must be considered, such as appropriate molecular descriptors for each regression model and steric effect.

The predictive ability of the four models was evaluated in terms of RMSEC. Applying four equations to predict oral absorption of the training set and test set, respectively. Fit error of models for all chemical drugs (training set, RMSEC = 7.76; test set, RMSEC = 5.06), acidic chemical drugs (training set RMSEC = 4.38; test set RMSEC = 3.95), basic chemical drugs (training set RMSEC = 3.08; test set RMSEC = 2.98) and natural chemical drugs (training set RMSEC = 3.00) were relative low and descend gradually one by one, which suggested the better predictive ability was obtained and ionization of chemical drugs was considered necessarily. Fig. 1a-4a (supplementary files) and Fig. 1b-3b (supplementary files) show the activities of predicted value vs. experimental value of training set and test set for four models respectively. As can be observed, the better correlations were obtained and the correlation between predicted value and experimental value was improved one by one.

Application developed regression methods for chemical drugs to predict oral absorption of bioactive ingredients of traditional Chinese medicines by biopartitioning micellar chromatography

The human intestinal absorption of drugs can be classified into three categories which are poor intestinal absorption ranged less than 30 %, high intestinal absorption ranged more

than 80 % and moderate intestinal absorption ranged between these two values 30 % and 80 %²⁸. The absorption in rats could be used as an alternative method to human absorption in pre-clinical oral absorption studies²⁹. In this paper, animal intestinal absorption accounts to human oral absorption.

The ionization effect of bioactive ingredients of traditional Chinese medicines is common with the chemical drugs in membrane permeation when these acidic and basic bioactive ingredients dissociate under physiological pH. On the basis of relationships between log k and oral absorption for chemical drugs as mentioned above, oral absorption % of traditional Chinese medicines for bioactive ingredients were evaluated.

Prediction oral absorption of organic acid of traditional Chinese medicines for bioactive ingredients: Established regression models for all chemical and acidic chemical drugs were used to predict oral absorption % of organic acids of traditional Chinese medicines for bioactive ingredients. Molecular descriptors and capacity factor (k) for selected organic acid of bioactive ingredients were shown in Table-3 (supplementary files). The predicted oral absorption % calculated separately by eqns. 1 and 2, and experimental oral absorption % from literatures were listed in Table-4 (supplementary files). It was found that most of bioactive ingredients of traditional Chinese medicines in oral absorption predicted by model of acidic chemical drugs were better agreement with literature reported value than predicted by model of all chemical drugs.

Molecular descriptors selected for these structures are appropriate or not, which can affect prediction accuracy. Moreover, experimental condition to oral absorption date obtained may influence the accuracy in oral absorption. Therefore, not all of the predicted values of organic acids of traditional Chinese medicines for bioactive ingredients were consistent with literature value and acidic chemical drugs prediction model was not always better than model of all chemical drugs in predictability. Caffeic acid³⁰ in the human body is passive diffusion through the deputy cells. Experiment founded that the oral absorption % of caffeic acid was 95 % obtained by orally administrated to healthy subjects. But its predicted oral absorption % by model of all chemical drugs was 79.56 and 70.09 % predicted by model of acidic chemical drugs. Both of predicted values were lower than reported value. Besides, bioactive ingredients of traditional Chinese medicines in oral absorption % dates have not been studied clearly, so some of them can not get exact oral absorption % value in this paper. For example, cinnamic acid, through models of acidic chemical drugs and all chemical drugs, the predictive value of cinnamic acid was 67.66 and 45.17 %, respectively. This can promote further study pharmacokinetics property of cinnamic acids and its analogues.

TABLE-3
DESCRIPTORS FOR ORGANIC ACIDIC OF TRADITIONAL CHINESE MEDICINES FOR BIOACTIVE INGREDIENTS

No.	Compound	$1/k_{BMC}$	NHDL	Kappa_2	NTR	K3A	JY	NAR	NSB	NH
1	Chlorogenic acid	0.39	6.00	8.79	1.00	4.56	2.09	1.00	1.00	18.00
2	Ferulaic acid	0.21	2.00	5.78	1.00	6.07	2.98	1.00	1.00	8.00
3	Caffeic acid	0.19	3.00	5.02	1.00	2.69	2.24	1.00	1.00	8.00
4	Cinnamic acid	0.16	5.00	6.79	1.00	3.78	1.74	1.00	1.00	8.00
5	Rhein	0.07	3.00	5.89	1.00	5.82	2.38	2.00	0.00	8.00
6	Salvianolic acid B	0.24	9.00	19.43	2.00	8.83	1.49	2.00	1.00	30.00
7	Tanshinol	0.39	7.00	12.19	2.00	6.88	2.81	1.00	0.00	9.00

TABLE-4
EXPERIMENTAL ORAL ABSORPTION % AND PREDICTED OA % OF ORGANIC ACIDIC OF
TRADITIONAL CHINESE MEDICINES FOR BIOACTIVE INGREDIENTS

No.	Compound	Predicted (oral absorption %)		Experimental (oral absorption %)
		By predictive model of all chemical drugs	By predictive model of acidic chemical drugs	
1	Caffeotannic acid	55.87	43.87	33.33 ^a
2	Ferulaic acid	82.46	53.72	56.1 ± 2.3 ^b
3	Caffeic acid	79.56	70.09	95.00 ^a
4	Cinnamic acid	67.66	45.17	–
5	Rhein	79.88	53.24	50-60 ^c
6	Salvianolic acid B	16.06	10.82	4.46-14.55 ^d
7	Tanshinol	36.22	25.02	7.74-17.75 ^d

^aReceived from literature M.R. Olthof, P.C.H. Hollman and M.B. Katan, *The Journal of Nutrition*, **131**, 66 (2001).

^bReceived from literature A. Adam, V. Crespy, M.A. Levrat-Verny, F. Leenhardt, M. Leuillet, C. Demigne and C. Remesy, *The Journal of Nutrition*, **132**, 1962(2002).

^cReceived from literature W. Lang, *Pharmacology*, **36**, Supp 1, 158 (1988).

^dReceived from literature Y.F. Zhang, Y.J. Li, Q. Yang, X.G. Weng, Y. Dong and X.X. Zhu, *Chinese Journal of Experimental Traditional Medical Formulae*, **16**, 96(2010).

TABLE-5
DESCRIPTORS FOR ALKALOIDS OF TRADITIONAL CHINESE MEDICINES FOR BIOACTIVE INGREDIENTS

No.	Compound	1/k _{BMC}	NHDL	Kappa_2	NTR	IC	MS	NR	PSA
1	Sinomenine	0.65	2.00	10.31	2.00	6.25	-6.72	0.00	109.00
2	Berberine	0.42	2.00	13.87	2.00	5.46	-5.15	1.00	170.79
3	Aconitine	0.08	2.00	7.39	0.00	4.44	-3.82	2.00	109.51
4	Hypaconitine	0.10	2.00	11.28	0.00	3.51	-3.73	2.00	133.21
5	Colchicine	0.79	1.00	10.54	1.00	3.93	-4.70	0.00	83.09
6	Theophylline	0.24	1.00	3.29	0.00	6.09	0.03	1.00	69.29
7	Tetrahydropalmatine	0.50	1.00	9.16	2.00	4.93	-5.49	0.00	70.16

TABLE-6
EXPERIMENTAL ORAL ABSORPTION % AND PREDICTED ORAL ABSORPTION % OF ALKALOIDS OF
TRADITIONAL CHINESE MEDICINES FOR BIOACTIVE INGREDIENTS

No.	Compound	Predicted (oral absorption %)		Experimental (oral absorption %)
		By predictive model of all chemical drugs	By predictive model of basic chemical drugs	
1	Sinomenine	58.41	25.82	Oral absorption % ≤ 30 ^a
2	Berberine	55.41	38.12	Oral absorption % ≤ 30 ^b
3	Aconitine	90.17	85.58	–
4	Hypaconitine	82.56	91.31	–
5	Colchicine	69.18	59.63	Oral bioavailability, 47±13 ^c
6	Theophylline	99.78	73.90	83 ± 4 ^d
7	Tetrahydropalmatine	67.52	45.43	40-50 ^d

^aReceived from literature W.Y. Chen, Y.D. Zhou, J.P. Kang, Q.B. Li and X.K. Fen, Study on absolute bioavailability of enteric-coated tablets of sinomenine. Chengyu Pharmaceutical Annual Conference Proceedings (2008) (in Chinese).

^bReceived from literature Y.W. Hua, Q.Y. Zhang, D. Li, J.C. He and G.L. Xu, *Journal Pharmaceutical and Clinical Research*, **17**, 465 (2009) (in Chinese).

^cReceived from literature G.M. Ferron, M. Rochdi, W.J. Jusko and J.M. Scherrmann, *Journal of Clinical Pharmacology*, **36**, 874 (1996).

^dReceived from literature Z.Y. Hong, G.R. Fan, Y.F. Chai, X.P. Yin and T.Yu. *Chinese Pharmaceutical Journal*, **42**, 1104 (2007) (in Chinese).

Prediction oral absorption of alkaloids of traditional Chinese medicines for bioactive ingredients: Models created by all chemical drugs and basic chemical drugs were used to predict oral absorption % of alkaloids of traditional Chinese medicines for bioactive ingredients. The molecular descriptors and value of capacity factor (k) for selected alkaloids are showed in Table-5 (supplementary files). The predicted oral absorption % calculated separately by eqns. 1 and 3, and experimental oral absorption % from literatures are showed in Table-6.

Due to poor oral absorption and inappropriate half-life time, most of bioactive ingredients of traditional Chinese medicines need to be orally administrated many times and choose

to be administrated through transdermal, intramuscular and intravenous. Adding to not clear research on the pharmacokinetics particular absorption process, most of alkaloids of traditional Chinese medicines for bioactive ingredients can not obtain available oral absorption %. For example, sinomenine and berberine have poor oral absorption. Tetrahydropalmatine absorbed in the rat small intestine was probable mainly passive diffusion. However, their oral absorption % predicted by model of basic chemical drug was better consistent with literature reported than model of all chemical drugs (Table-6 in supplementary files). Oral bioavailability is particularly important for lead optimization considering the importance of developing

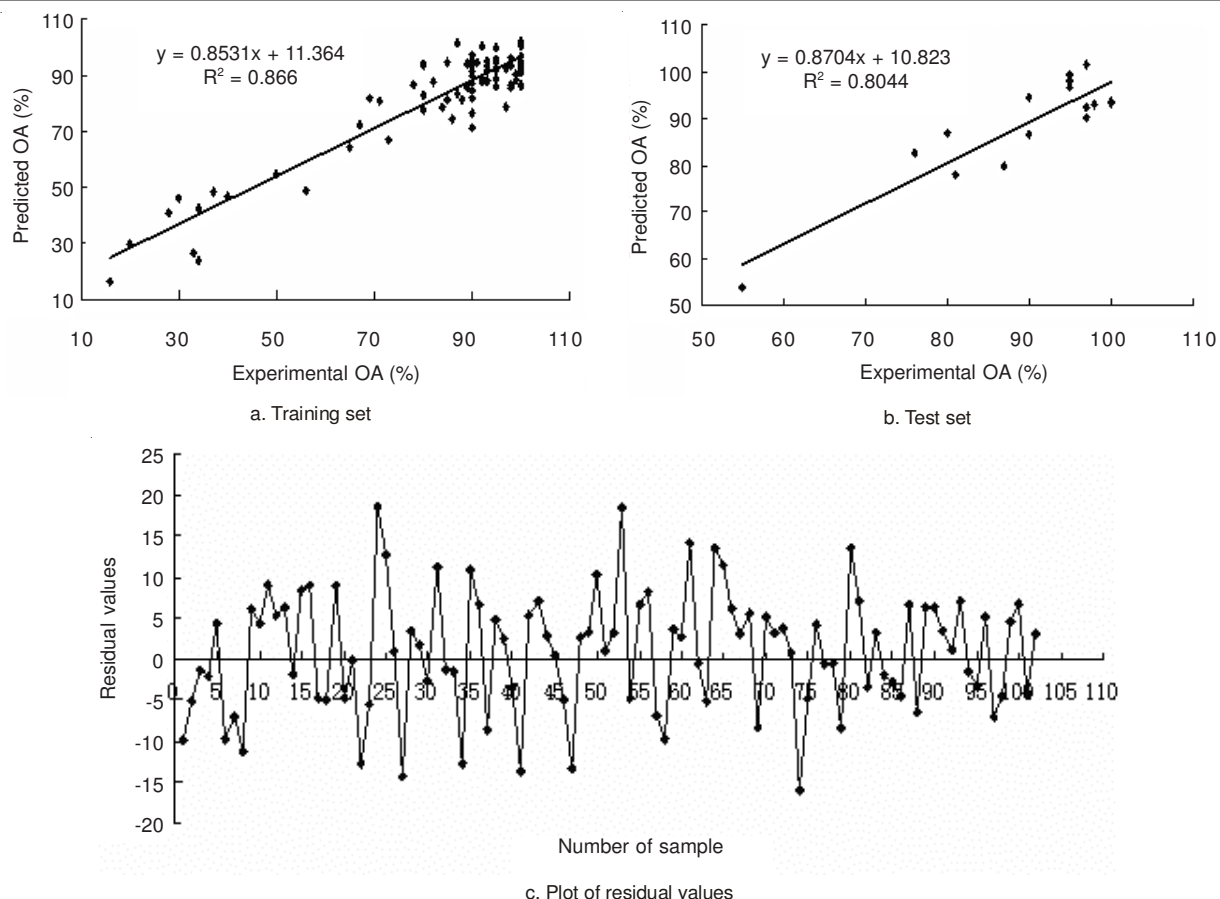


Fig. 1. (a) and (b) show that predicted value calculated by eqn. 1 vs. experimental value of training set and test set of all chemical drugs, respectively. c represents the residual values [Experimental oral absorption (%) - Predicted oral absorption (%)] of all chemical drugs, a, b and c are to validate the predicted ability of model constructed

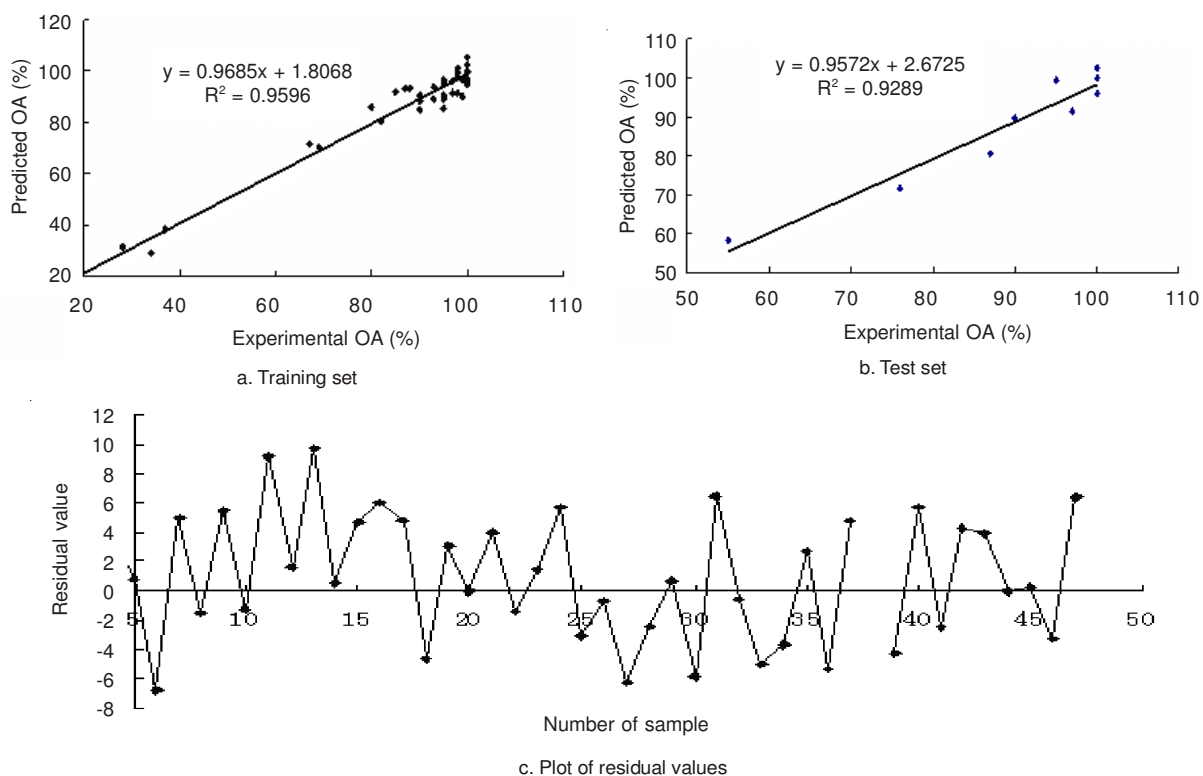


Fig. 2. (a) and (b) show that predicted value calculated by eqn. 2 vs. experimental value of training set and test set of acidic chemical drugs, respectively. c represents the residual values [Experimental oral absorption (%) - Predicted oral absorption (%)] of acidic chemical drugs, a, b and c are to validate the predicted ability of model constructed

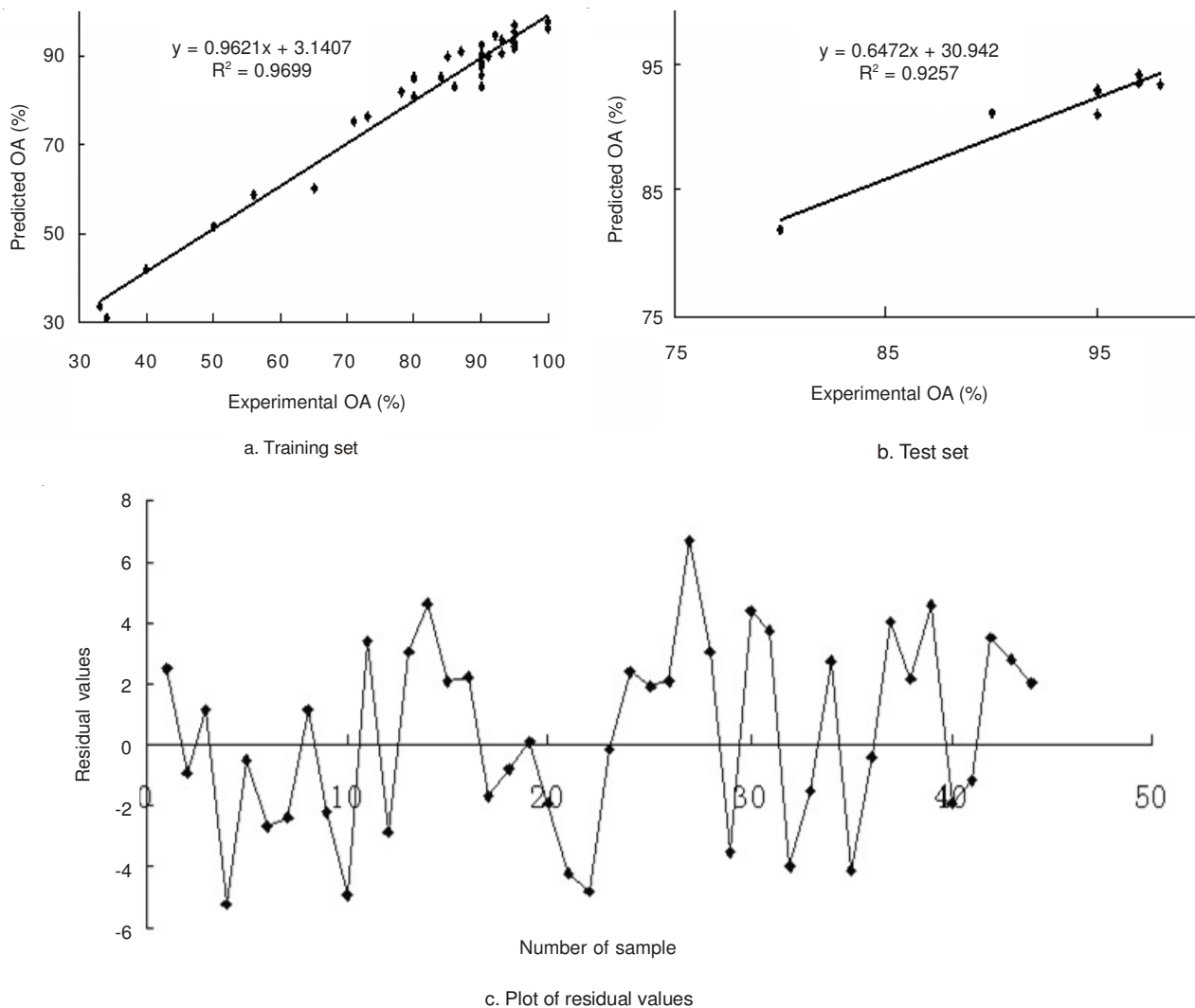


Fig. 3. (a) and (b) show that predicted value calculated by eqn. 3 vs. experimental value of training set and test set of basic chemical drugs, respectively. c represents the residual values [Experimental oral absorption (%) - Predicted oral absorption (%)] of basic chemical drugs, a, b and c are to validate the predicted ability of model constructed

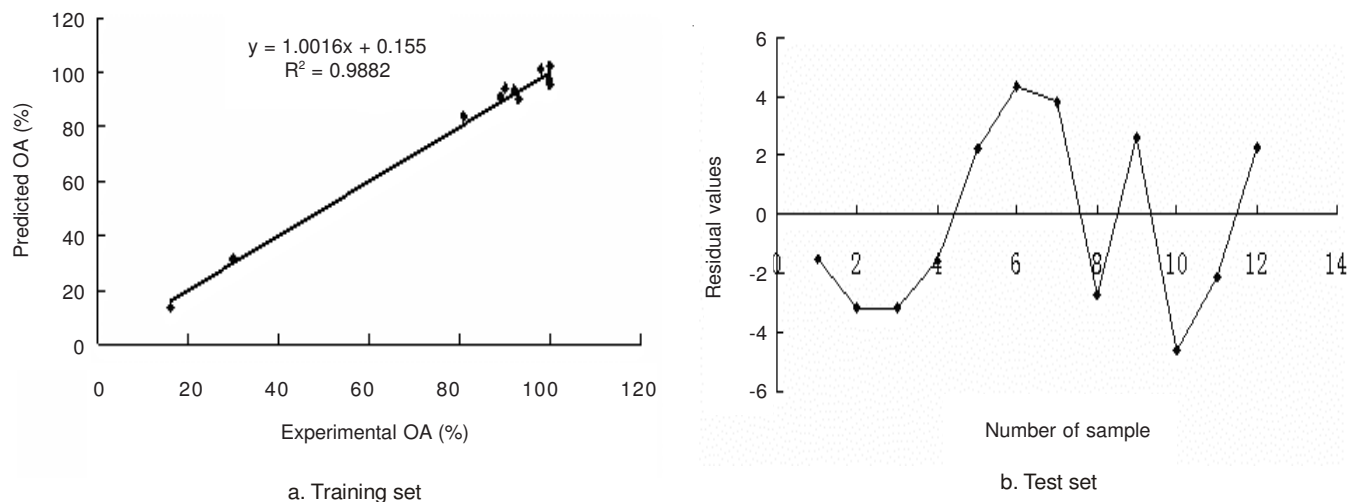


Fig. 4. (a) shows that predicted value calculated by eqn. 4 vs. experimental value of training set natural chemical drugs, respectively. (b) represents the residual values [Experimental oral absorption (%) - Predicted oral absorption (%)] of natural chemical drugs, a and b are to validate the predicted ability of model constructed

orally administered commercial products. After oral administration, the bioavailability was low, the absorption should be equal to or higher than the values of bioavailability because following absorption from the gastrointestinal tract, the drug passes directly to the liver *via* the hepatic portal vein where it may be extensively metabolized before reaching the systemic circulation³¹, such as berberine and colchicines³². The literature just reported hydrochloride bioavailability of berberine was about 10%. Colchicine belongs to the class of moderate oral absorption which ranges from 30 to 80%. Its oral bioavailability was about 40-50%. Their oral absorption % prediction values were higher than their oral bioavailability reported value (Table-6) and predicted value from model of basic chemical were more close to reported value than model of all chemical drugs. Similarly, prediction from basic chemical drugs model was more consistent with literature reported.

Aconite root is mainly composed of aconitine and hypaconitine that are diester diterpenoid alkaloids and the nature are unstable, decomposing in the water immediately. Previous studies³³ applied Caco-2 cell model to simulate the human body's intestinal absorption process, predict the oral absorption of chemicals and use this model to carry out study of absorption for aconitine and hypaconitine. The research revealed that the apparent permeability coefficients (P_{app}) value of aconitine and hypaconitine have good oral absorption because their P_{app} values were more than 1×10^{-6} cm/s. P_{app} which associates with the oral absorption % of chemical drugs directly reflects the drug absorption. There is complete absorption when P_{app} value of drug is large than 1×10^{-6} cm/s³⁴. Predicted oral absorption % of aconitine by models of all chemical drugs and basic chemical drugs was 90.17 and 85.58% and hypaconitine was 82.56 and 91.31%.

Conclusion

In this study, when the acid-base property of drugs was taken into consideration to predict oral absorption of diverse structural drugs by biopartitioning micellar chromatography technique, capacity factor (k_{BMC}) measured on biopartitioning micellar chromatography column at pH 7.4 and molecular structure descriptors were better correlated with oral absorption % of drugs. At the same time, constructed models process certainly fit ability and statistics significance. The absorption model allows prediction of oral absorption can be used to determine how to modify drug structure to improve absorption. In summary, four prediction models of chemical drugs constructed are robust and reliable. So application those in prediction oral absorption of monomers of active ingredients in traditional Chinese medicines are practical.

We just preliminarily studied retention on the biopartitioning micellar chromatography and molecular descriptors of monomers of traditional Chinese medicines for bioactive ingredient through model of chemical drugs to predict oral absorption. However, absorption is a complex kinetic process that is dependent on numerous biochemical, physiological and physico-chemical factors. Experimental error for obtaining absorption value is quite high, especially for low absorption. It is unrealistic to attempt to obtain prediction bioactive values which agree well with reported values which were obtained from animals. So large data set and complete molecular

descriptors and accurate literature value about bioactive should be considered when construct prediction model.

In following research, we aim to further study oral absorption. To mimic an environment, which more closely resembles the conditions encountered as the substance moves through the gastrointestinal tract and knowing the importance of pH conditions to retention of acidic compounds, capacity factor measurements will be made at varying pH values, from pH 4.5-7.4. And appropriate molecular descriptors of different aspects will be selected to construct model and evaluate predictive ability by RMSEC (the root-mean-square error of calibration) and RMSECV (root-mean-square error of cross-validation). Besides, screening bioactive ingredients from traditional Chinese herbal medicine will apply high throughput screening method biopartitioning micellar chromatography by means of combinatorial chemistry which are widely used in drug research and discovery in conjunction with mathematical statistics method to construct QRRAR/QSAR models to predict oral absorption, distribution, metabolism and elimination (ADME) of traditional Chinese medicine compound preparation.

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